



IMPERIAL INSTITUTE  
OF  
AGRICULTURAL RESEARCH, PUSA.







**THE ANNALS OF  
APPLIED BIOLOGY**

CAMBRIDGE UNIVERSITY PRESS  
LONDON: FETTER LANE, E.C. 4



H. K. LEWIS & CO., LTD., 136, GOWER STREET, LONDON, W C. 1  
PARIS: LIBRAIRIE HACHETTE & CIE.  
CHICAGO: THE UNIVERSITY OF CHICAGO PRESS  
(Agents for the United States)  
BOMBAY, CALCUTTA, MADRAS: MACMILLAN & CO., LTD.  
TOKYO: THE MARUZEN-KABUSHIKI-KAISHA

*All rights reserved*

# THE ANNALS OF APPLIED BIOLOGY

EDITED BY

W. B. BRIERLEY

AND

D. WARD CUTLER

VOLUME XVI

CAMBRIDGE

AT THE UNIVERSITY PRESS

1929

PRINTED IN GREAT BRITAIN

# CONTENTS

NO. 1 (FEBRUARY, 1929)

	PAGE
1. Studies on Potato Virus Diseases. IV. Further Experiments with Potato Mosaic. By KENNETH M. SMITH, D.Sc. (With Plates I-V) . . . . .	1
2. On Two Cases of Recovery from a Mosaic Disease of Tomato Plants, <i>Lycopersicon esculentum</i> . By LEN VERWOERD, M.Sc. Agric. (Stell.) . . . . .	34
3. Studies of Wood-Destroying Fungi. I. <i>Polyporus hispidus</i> (Fries). By F. J. NUTMAN, A.R.C.S., B.Sc. (With Plates VI-VIII and 2 Text-figures) . . . . .	40
4. The Biology of Oat Smuts. II. Varietal Resistance. By KATHLEEN SAMPSON, M.Sc. (Lond.). (With Plate IX and 1 Text-figure) . . . . .	65
5. The Control of "Bunt" in Wheat. By W. A. R. DILLON WESTON, M.A. . . . .	86
6. The Action of Sulphur as a Fungicide and as an Acaricide. Part II. By WM. GOODWIN and H. MARTIN. (With 1 Text-figure) . . . . .	93
7. On the Occurrence of the Parthenogenetic and Sexual Forms in <i>Aphis rumicis</i> L., with Special Reference to the Influence of Environmental Factors. By J. DAVIDSON, D.Sc. (With 6 Text-figures) . . . . .	104
8. Studies on <i>Oscinella frit</i> Linn. A Report on certain Oat Varieties in Relation to their Resistance to Attack by the Frit Fly in Sweden, together with Data concerning the Production of Resistant Utility Varieties. By NORMAN CUNLIFFE, M.A. (With 8 Charts) . . . . .	135
9. Note on the Growth of Young Mice Suckled by Rats. By A. S. PARKES, D.Sc. . . . .	171
10. Reviews . . . . .	174
11. Proceedings of the Association of Economic Biologists. . . . .	181
12. List of Members of the Association of Economic Biologists, 1928 . . . . .	195
13. Laws of the Association of Economic Biologists . . . . .	204

## NO. 2 (MAY, 1929)

1. Studies on Potato Virus Diseases. V. Insect Transmission of Potato Leaf-Roll. By KENNETH M. SMITH, D.Sc. (With Plates X-XII)	209
2. Investigation of Hop Mosaic Disease in the Field. By W. F. CHEAL. (With 2 Text-figures)	230
3. Observations of the <i>Helminthosporium</i> Diseases of Cereals in Britain. I. The Behaviour of <i>Helminthosporium gramineum</i> in a Common Barley Disease. By NOEL J. G. SMITH, Ph.D. (Cantab.). (With 3 Text-figures)	236
4. On the Stem Root or Wilt Disease of Carnations. By W. J. DOWSON, M.A., D.Sc. (With Plate XIII)	261
5. The Influence of Bright Sunshine upon the Tomato under Glass. By W. F. BEWLEY, D.Sc. (With 5 Text-figures)	281
6. The Biology of Thysanoptera with Reference to the Cotton Plant. IV. The Relation between the Degree of Infestation and Surface Caking of the Soil. By ELSIE I. MACGILL, M.Sc. (With 2 Text-figures)	288
7. A Recording Scale for Bee Hives. By D. M. T. MORLAND, M.A. (With 4 Text-figures)	294
8. A Survey of the Insect and other Invertebrate Fauna of Permanent Pasture and Arable Land of certain Soil Types at Aberystwyth. By E. E. EDWARDS, M.Sc. (With 4 Text-figures)	299
9. Investigations on <i>Heterodera schachtii</i> in Lancashire and Cheshire. Part I. The Infestation in certain Areas as revealed by Cyst Counts; an Estimation of the Errors involved in the Technique and a Correlation with Intensity of Disease. By A. M. SMITH, B.Sc., Ph.D., A.I.C. and E. G. PRENTICE, B.Sc. (With 4 Text-figures)	324
10. Investigations on <i>Heterodera schachtii</i> in Lancashire and Cheshire. Part II. The Relationships between Degree of Infestation and Hygroscopic Moisture, Loss on Ignition and pH Value of the Soil. By A. M. SMITH, B.Sc., Ph.D., A.I.C. (With 2 Text-figures)	340
11. Reviews	347

	PAGE
12. Report of the Council of the Association of Economic Biologists for the year 1928 . . . . .	355
13. Report of the Honorary Treasurer for the year 1928 . . . . .	356
14. Balance Sheet of the Association of Economic Biologists . . . . .	357

No. 3 (AUGUST, 1929)

1. The Mosaic Disease of the Hop; Grafting Experiments, II. By D. MACKENZIE, E. S. SALMON, W. M. WARE and R. WILLIAMS . . . . .	359
2. Studies on Potato Virus Diseases. VI. Further Experiments with the Virus of a Potato Mosaic upon the Tobacco Plant. By KENNETH M. SMITH, D.Sc. (With Plates XIV-XVII and 1 Text-figure) . . . . .	382
3. <i>Daldinia concentrica</i> attacking the Wood of <i>Fraxinus excelsior</i> . By THÉRÈSE E. PANISSET, A.R.C.S., B.Sc. (With 23 Text-figures) . . . . .	400
4. Additional Hosts of <i>Synchytrium endobioticum</i> (Schilb.) Perc. By MARY S. MARTIN, B.Sc. (With Plates XVIII and XIX) . . . . .	422
5. The Effect of Copper Sulphate on Tomato Plants. By O. OWEN, M.Sc., Ph.D., A.I.C. . . . .	430
6. Some Properties of the Cell-Wall of Cotton-Hairs. By N. W. BARRITT, M.A. (With 3 Text-figures) . . . . .	438
7. The Production of Ethyl Alcohol and Acetaldehyde by Apples in Relation to the Injuries occurring in Storage. Part I. Injuries to Apples occurring in the Absence of Oxygen and in certain Mixtures of Carbon Dioxide and Oxygen. By MEIRION THOMAS, M.A. (With Plate XX) . . . . .	444
8. The Internal Condition of the Host Plant in Relation to Insect Attack, with Special Reference to the Influence of Pyridine. By J. DAVIDSON, D.Sc. and H. HENSON, B.Sc. . . . .	458
9. Carbon Dioxide Production in Sands and Soils in the Presence and Absence of Amoebae. By D. WARD CUTLER, M.A. and L. M. CRUMP, M.Sc. (With 2 Text-figures) . . . . .	472
10. Obituary Notice. Professor J. RITZEMA BOS, 1850-1928. (With Plate XXI) . . . . .	483
11. Reviews . . . . .	486
12. Proceedings of the Association of Economic Biologists. . . . .	490



## No. 4 (NOVEMBER, 1929)

PAGE

1. Manganese as an Essential Element for Plant Growth.  
By GEOFFREY SAMUEL (Plant Pathologist) and C. S. PIPER  
(Chemist). (With Plates XXII-XXIV and 2 Text-figures) . . . 493
2. A Mosaic Virus of Grasses, not Virulent to Sugar Cane.  
By H. H. STOREY, M.A., Ph.D. . . . . 525
3. Observations during 1927-28 on the Incidence of "Rusts"  
on various selected Wheat Varieties, with Special Reference to  
the Intensity of Yellow Rust, *Puccinia glumarum*, Eriks. and  
Henn. By W. A. R. DILLON WESTON, M.A. . . . . 533
4. Treatment of Sugar Beet "Seed" to prevent Seedling  
Diseases. By R. C. WOODWARD, B.Sc., Ph.D. and W. A. R.  
DILLON WESTON, M.A. (With Plate XXV and 3 Text-figures) . . . 542
5. Studies in Bacteriosis. XVI. The Agglutinating and  
Plasmolytic Action of the Sap of the Potato on various Para-  
sitic and Saprophytic Species of Bacteria. By EMILY M.  
BERRIDGE, D.Sc. (With 2 Text-figures) . . . . . 567
6. The Morphology and Physiology of Two Lactose-fer-  
menting Yeasts and Chemical Changes during the Ripening  
of Cheese from Milk containing them. By L. A. ALLEN, B.Sc.  
and B. D. THORNLEY, B.Sc. (With 4 Text-figures). . . . . 578
7. Investigations on *Heterodera schachtii*, Schmidt. in  
Lancashire and Cheshire. Part III. Certain Correlations  
between Crop Yields and Degree of Infestation. By A. M.  
SMITH, B.Sc., Ph.D., A.I.C. and HERBERT W. MILES, M.Sc.,  
N.D.A. (With 1 Text-figure) . . . . . 596
8. Pollination of Hardy Fruits: Insect Visitors to Fruit  
Blossoms. By G. FOX WILSON. (With 1 Text-figure) . . . . 602
9. The Larva and Pupa of *Scatopse fuscipes* Mg. and a Com-  
parison of the known Species of Scatopsid Larvae. By EDITH  
LYALL, B.Sc. (With 14 Text-figures) . . . . . 630
10. Reviews . . . . . 643

## STUDIES ON POTATO VIRUS DISEASES

## IV. FURTHER EXPERIMENTS WITH POTATO MOSAIC

By KENNETH M. SMITH, D.Sc.

*(Potato Virus Research Station, School of Agriculture, Cambridge.)*

(With Plates I-V.)

## CONTENTS.

	PAGE
1. INTRODUCTION . . . . .	1
2. MATERIAL AND METHODS . . . . .	2
3. EXPERIMENTAL TRANSMISSION OF POTATO MOSAIC BY INSECTS . . . . .	3
(a) Haulm infections, 1926 . . . . .	3
(b) Sprout infections, 1927 . . . . .	5
4. ATTEMPTED TRANSMISSION OF POTATO MOSAIC BY MEANS OF INOCULATION OF HEALTHY POTATOES WITH THE TISSUES OF PRESUMABLY INFECTIVE INSECTS .	6
5. EXPERIMENTAL TRANSMISSION OF THE VIRUS OF POTATO MOSAIC BETWEEN POTATO AND TOBACCO PLANTS . . . . .	7
6. DETAILS OF EXPERIMENTS . . . . .	23
7. DISCUSSION . . . . .	28
8. SUMMARY . . . . .	30
9. REFERENCES . . . . .	32
EXPLANATION OF PLATES . . . . .	33

## I. INTRODUCTION.

THE fact that some at least of the potato virus diseases are spread from plant to plant through the agency of insects is no longer open to doubt, but information as to which of the many insect species attacking the potato are capable of disseminating the virus is scanty. Some evidence has already been presented by the writer<sup>(8)</sup> that the most efficient carrier of mosaic disease in Great Britain is the aphid *Myzus persicae* Sulz. and possibly also another species of aphid, *Macrosiphum gei* Koch. Further evidence of successful transmission by the former aphid is offered in the ensuing communication, together with an account of some negative experiments with other potato insects.

The main purport of this paper, however, is to describe in detail experiments with the virus of potato mosaic upon another Solanaceous

host, ~~i.e. the~~ tobacco plant, illustrating the disease it produces in that host, and the changes that have occurred in the response of the potato plant to the mosaic virus after its passage through the tobacco plant.

Grateful acknowledgment is due to Miss F. E. Hawkes, who by her entirely voluntary assistance in the mornings during the summer of 1928 relieved the writer of much necessary routine work.

## 2. MATERIAL AND METHODS.

### *Technique of insect infection of potato and tobacco plants.*

(a) *Potato.* With the exception of the insect infections of the potato haulm performed in 1926 in the open under insect-proof cages, all the work detailed in this communication was carried out in the insect-proof glass-houses at the Potato Virus Research Station, School of Agriculture, Cambridge.

To infect the insects used with the mosaic virus they were colonised upon mosaic potato plants growing in pots, either under large glass cylinders with canvas tops or in rectangular wooden cages also with canvas tops and with glass in three out of the four sides. For aphid infection of the haulms of healthy potatoes the same type of cage was used, the bottoms of the cages being covered with sand as an additional safeguard against insect escape. For insect infection of the sprouted half tuber, two methods of procedure were tried. The first method consisted in placing the sprouted half tuber in an ordinary two-pound glass jam jar, fitted with a muslin top secured with a rubber band, and introducing the insects on to the sprouts. This was found unsatisfactory and soon abandoned, as the half tuber tended either to rot or dry up and the sprouts thus to become unsuitable for the insects to feed upon them. The second method of sprout infection was definitely satisfactory, and consisted in planting the half tuber in a pot with the sprouts uncovered by soil, a glass lamp chimney of the "hurricane" or stable lamp type was then placed over the sprouts and the insects introduced.

The rim of the lamp glass allowed of a canvas top, held in place by a rubber band, being fixed, while the lower rim of the glass was pressed into the soil, thus providing an insect-proof cage. By this method ideal conditions for plant infection were obtained, *i.e.* a young and rapidly growing plant, together with the necessary humidity for the insect, an important point in the case of the aphid. After the requisite time for infection of the plant had elapsed, the lamp chimney was removed, the young shoot sprayed with nicotine and soap and allowed to grow to maturity.

(b) *Tobacco*. The tobacco plants, used either as the source of infection or as healthy plants to be infected, were grown in pots under the glass cylinders and glass-sided cages already mentioned. It was necessary to use small seedlings, both for ease of infection and because the later development of sticky hairs prevented the colonisation of the aphides (the only insects so far used in tobacco infection). After the necessary time had elapsed the plants were sprayed with nicotine and soap.

*Origin of plants used.*

The healthy potato plants were mostly of two varieties, Arran Victory and President. The former were part of the writer's healthy stock, grown for 2 to 3 years under insect-proof conditions, and the latter were tested tubers kindly given by Dr R. N. Salaman. The mosaic potato plants were of the same two varieties, and had also been grown for 2 years under the same conditions.

The tobacco plants were of two varieties, White Burley and Virginia, grown from seed supplied by Messrs Sutton of Reading.

*Origin of insects used.*

The aphid, *Myzus persicae*, so largely used in these experiments, was from a stock which had been breeding continuously for three years upon non-Solanaceous plant hosts such as spinach, radish, cabbage and chrysanthemum, etc. The other insects used were collected mostly from nettle which is an alternate host for the majority of potato-inhabiting insects.

### 3. EXPERIMENTAL TRANSMISSION OF POTATO MOSAIC BY INSECTS.

(a) *Haulm infections, 1926.*

Two plots of land near Manchester were used for these experiments, one in the experimental grounds of the University, and the other in the grounds of the Shirley Institute at Didsbury, by kind permission of the director. The plants used in one plot were part of the writer's stock of healthy Arran Victory, and those used in the other were Tinwald Perfection, Scotch "seed." Each healthy tuber used in these experiments was divided into two halves, which were planted under separate and similar insect-proof cages which have been described elsewhere (8). One half formed the plant to be infected, and the other half acted as a control, two plants per insect species were used in each plot. When the experimental plants were about 10 in. high they were colonised with the various insects under test, which had previously fed for a minimum

period of a week upon a mosaic potato plant, variety President. A separate mosaic plant was used to infect each species of insect. The following insects were used:

### HEMIPTERA

#### Capsidae

*Lygus pabulinus* Linn.

*Calocoris bipunctatus* Fab.  
(*norvegicus*)

#### Aphididae

*Myzus persicae* Sulz.

*Macrosiphum gei* Koch.

*Myzus circumflexus* Buckt.

#### Leaf-hoppers

*Zygina pallidifrons* Edw.

*Eupteryx auratus* Liv.

#### Aleurodidae

*Asterochiton vaporariorum* Westw.

### COLEOPTERA

*Psylliodes affinis*.

All these insects were successfully colonised, and the plants grew normally with the exception of the two Arran Victory potatoes colonised with the aphid *Macrosiphum gei*, which died down prematurely.

*Results of 1926 haulm infections.* All the plants in Exp. 1 (Arran Victory) with their controls remained healthy during 1926. Exp. 2 (Tinwald Perfection) had to be discarded owing to the development of mosaic in the control plants. The tubers resulting from the Arran Victory experiment were carefully harvested from both experimental and control plants and kept in glass jars with close-fitting canvas tops until the spring of 1927, when they were brought to Cambridge and planted in the insect-proof glass-house of the Potato Virus Research Station. The progeny of both experimental and control plants remained healthy throughout 1927. The tubers resulting from these plants were harvested and planted under the same conditions as in 1927. This time mosaic of a well-marked type developed in all the plants originating from one of the potatoes colonised with *Myzus persicae* in 1926. The controls to this experiment and all the other insect experiments and controls remained healthy. Although some tubers were formed, the experiment with the aphid *M. gei* cannot be considered owing to the premature failure of the haulms. It would appear from the results of this experiment that it is possible for the mosaic virus to lie latent, at any rate in the variety Arran Victory. There seems no explanation other than that the aphid *M. persicae* had infected the plant in 1926 and the virus had remained dormant till 1928, as there had been no chance of any extraneous infection between that time and the development of mosaic symptoms.

That the aphid, *M. persicae*, does actually pick up the virus of potato mosaic with regularity has been proved by the writer, and will be dealt with later in this paper. Although it must be regarded as a possibility, the phenomenon of temperature masking hardly seems adequate as an explanation of this belated development of mosaic, as other plants under exactly similar conditions showed the mosaic symptoms quite normally. As the progeny of the plants, colonised in 1926 with the other species of insects, remained healthy throughout 1928, it would appear that these insects had failed to transmit the virus. After several years' experience with potatoes under insect-proof cages the writer has formed the opinion that absolutely critical experimental work with the insect transmission of potato virus diseases cannot be performed under cages out of doors, and this type of experiment has therefore been abandoned.

(b) *Sprout infections, 1927.*

The experiments outlined in the preceding paragraphs were repeated in 1927 using the same insects, all the work being carried out in the insect-proof greenhouse. In this case, however, sprouted half tubers were used, and the insects, after feeding upon a mosaic Arran Victory plant, were colonised on the sprouts instead of on the growing plant. It was hoped thereby that the symptoms of mosaic disease, if transmitted, would show in the current season. The sprouted half tubers, after infection with the insects, together with the controls, were grown to maturity in the glass-house. All remained healthy with the exception of a certain percentage, the controls of which also developed mosaic: these were immediately discarded.

Owing to a shortage of healthy tubers the writer was compelled to perform some of the experiments with "seed" tubers bought from Scotland, but from this and other experiences it is not considered possible to perform experiments with potato mosaic except with tubers, the history of which is accurately known.

The progeny of the other experimental plants were grown in 1928, again with negative results, except in the case of *M. persicae*, where three plants out of twelve showed mosaic symptoms.

In this series of sprout infections many factors were varied, such as the times on source of infection and healthy sprout, temperature, and age of insect used, such as adult and larval forms.

4. ATTEMPTED TRANSMISSION OF POTATO MOSAIC BY MEANS OF INOCULATION OF HEALTHY POTATOES WITH THE TISSUES OF PRESUMABLY INFECTIVE INSECTS.

*Exp. 1.* Attempted infection of healthy potato plants by means of the body juices of mosaic-carrying aphides.

About 8 c.c. in bulk of the aphids, *M. persicae*, which had lived only upon a mosaic potato plant (Arran Victory) were triturated *en masse* in a mortar, under sterile conditions, with 20 c.c. of sterile water. The resulting fluid was filtered through a piece of fine muslin to remove the insect fragments, and some of the clear fluid thus obtained was apportioned into six small sterile glass tubes. Shoots were cut from six known healthy potato plants, Arran Victory and President, and placed each in a tube containing the aphid extract for 48 hours. At the end of that time each shoot had absorbed practically all the fluid in its tube. The shoots were then grafted back each on to its own plant. Union was made in every case and the shoots grew normally, no symptoms of mosaic developing upon either shoot or plant. A number of other healthy shoots were allowed to absorb the remaining aphid extract and were then plunged in pots where they put out roots and made plants. These remained healthy. The progeny of all the plants used in the above experiment were grown the following year and all produced healthy plants.

*Exp. 2.* Insertion into healthy potato sprouts of the salivary glands of two species of Capsid bugs (*Lygus pabulinus* and *Calocoris bipunctatus*) which had been bred upon mosaic potato plants.

These experiments were carried out during the two years 1926-1927. In the first year the salivary glands of twenty-five specimens of each of the two species of Capsid bugs were extracted and inserted with a needle into the sprouts of twelve healthy half tubers, each with a half tuber control. The half tubers were then potted up and grown under insect-proof conditions. This experiment was entirely negative, both inoculated and control half tubers producing healthy plants. It is noteworthy that the salivary glands of these two bugs produced necrotic lesions in the sprouts at the point of insertion.

In 1927 the procedure was altered slightly. The salivary glands of the two species of bugs which had fed, as before, on mosaic potato plants were extracted and triturated in a mortar under sterile conditions with 5 c.c. of sterile water. Shoots from known healthy Arran Victory and President plants were cut off and allowed to suck up this extract as in *Exp. 1.* They were then grafted back on to their respective plants, where they grew normally. No sign of mosaic appeared either in the plant itself

or on the grafted shoot, nor did the resulting tubers produce diseased plants when grown the following year.

From these two experiments, and others not described, it would appear impossible to infect potato plants with mosaic disease by means of inoculation with the body juices or salivary glands of insects which have been bred upon mosaic potato plants.

#### 5. EXPERIMENTAL TRANSMISSION OF THE VIRUS OF POTATO MOSAIC BETWEEN POTATO AND TOBACCO PLANTS.

After so much negative work with the attempted transmission of potato mosaic by means of insects, it occurred to the writer that if some other Solanaceous plant could be found which might more easily respond to the mosaic virus some further information might be obtained. If such a plant were infected it would at least act as an indicator in the sense that it would show whether or not the aphid was actually picking up the virus of potato mosaic. The plant selected for this work was the tobacco, two varieties being used, White Burley and Virginia. The following preliminary experiment which consisted of three parts was performed. Firstly, twelve half tubers of healthy Arran Victory, which were potted up and showing sprouts about 2 in. above the soil, were colonised with the aphid *M. persicae* which had been bred upon a mosaic Arran Victory plant. This aphid was chosen, judging from the writer's experience, as being the most likely insect to give positive results. Secondly, aphides from the same mosaic Arran Victory plant were colonised on six young tobacco plants, three White Burley, three Virginia. The third part of the experiment consisted of inoculations by means of a sterile needle, with juice from the same mosaic potato, on to six tobacco plants, three of each variety. The results of the experiment were as follows: the twelve Arran Victory potato plants showed no symptoms and were presumably still uninfected, the three White Burley tobacco plants colonised with the aphides developed a very marked spotting combined with mottling, the three Virginia plants colonised with the aphid developed no symptoms, while five out of the six tobacco plants inoculated by means of the needle developed some rather remarkable symptoms. Two out of the three Virginias showed a number of well-marked concentric rings, each with a central spot (Plate I, fig. 1), the walls of the rings being sharply cut, necrotic, and whitish in colour. The manifestations of this "ringspot" disease consisted either of two concentric rings, two pairs of concentric rings (Plate I, fig. 1), with a central spot, or sometimes single rings only without the spot. The three White Burley tobaccos showed a disease similar in general appearance,



but differing in the fact that instead of sharply cut concentric rings, the disease showed itself in the form of large numbers of round necrotic spots, some of which approached a ring-like form but seldom showing the very clear-cut rings appearing in Virginia.

This experiment was repeated with the tobaccos only, leaving out the aphid infection of the healthy Arran Victory plants, and using both aphides and inoculum from the original mosaic Arran Victory. The results of this second experiment were the same as those in the first.

From these two preliminary experiments a number of lines of enquiry were developed, the results of which, together with details of the experiments performed, are presented in the remainder of this paper.

To avoid the confusion likely to arise in describing numbers of cross-inoculation studies, the work has been grouped into the following sections, each series of experiments being dealt with separately.

*Section 1.* Inoculation of healthy tobacco plants of two varieties with the virus of potato mosaic.

(a) By needle.

(b) By aphid.

*Section 2.* Inoculation of healthy potato plants with the virus of various forms of tobacco ringspot.

(a) By needle.

(b) By aphid.

*Section 3.* Are the varying symptoms produced in tobacco by needle inoculation with potato mosaic manifestations of the same disease?

*Section 4.* Comparison of the symptoms produced in tobacco by aphid and needle inoculation respectively from the same mosaic potato.

*Section 5.* Transmission of tobacco ringspot to healthy tobacco.

(a) By needle.

(b) By aphid.

*Section 6.* Transmission of potato "ringspot" or intensified mosaic to healthy potato plants.

(a) By needle.

(b) By grafting.

(c) By aphid.

*Section 7.* What is the effect on the virus of tobacco ringspot of progressive inoculations through successive generations of tobacco plants?

*Section 8.* What is the effect on the virus of potato "ringspot" or intensified mosaic of progressive inoculations through successive generations of potato plants?

*Section 9.* Inoculation of healthy tobacco plants with the virus of potato "ringspot" or intensified mosaic.

(a) By needle.

(b) By aphid.

*Section 10.* Inoculation of healthy tobacco plants with the juice of known healthy Arran Victory potatoes.

*Section 11.* Effect of temperature on the symptoms of ringspot.

(a) In potato.

(b) In tobacco.

Filtration of the virus.

*Section 12.* Inoculation of tobacco plants with virus combinations of which potato mosaic is a constituent part.

*Section 13.* Needle inoculation of plants other than tobacco or potato with the virus of tobacco ringspot.

(a) Solanaceae.

(b) Other plants.

## SECTION 1.

Inoculation of healthy tobacco plants of two varieties with the virus of potato mosaic:

(a) *By needle.*

The mosaic virus was obtained from infected potatoes mostly of the variety Arran Victory, the history of which was known. These potatoes had been grown for two years under insect-proof conditions, and so far as could be ascertained were affected only with mosaic, the symptoms being the usual mottling associated with that disease. Leaves from such potatoes were ground up in a mortar under sterile conditions without the addition of water or with just sufficient to form a thick fluid of the consistency to allow of easy handling with a needle. This fluid was then scratched into the lamina and petiole of the tobacco leaf, while an incision was also usually made in the stem and some mosaic tissue inserted. It is important to use very small seedlings as it was found much more difficult to infect larger plants or those which were in any degree pot-bound.

*Development of ringspot in the inoculated tobacco plants.*

(a) *Virginia.* The development of symptoms of ringspot in both Virginia and White Burley is not always constant, even differing in a number of plants treated under identical conditions with the same inoculum. As a general rule, however, a mottling develops as the young leaves grow; this may disappear later, its place being taken by the curious

rings which give the disease its name, or it may persist in a characteristic spot-necrosis form. These rings in this particular variety may be of two kinds, either of a nebulous type somewhat suggestive of the watermark in a sheet of paper (Plate I, fig. 4) or of the concentric double ring with or without a central spot, and with clearly cut whitish necrotic walls (Plate I, fig. 1). The nebulous type of ring often appears first, later its place may be taken by the concentric ring, which is especially characteristic of Virginia tobacco, or the two may exist together. In one case the same inoculum produced the necrotic ring in one plant and the nebulous ring in another. As a rule, however, the necrotic concentric ring is the chief and may be the only symptom in this variety. Very often an infected Virginia plant presents a normal appearance except that in the centre of one leaf may appear a single concentric ring.

As the infected Virginia plant grows, new rings form on the young leaves; the old rings tend to lose their clear-cut character and to merge into necrotic spots, or they may persist for a time in the form of rings with reddish necrotic walls. When the plant is full grown and after the elapse of a month or two from the date of inoculation, the rings have mostly disappeared and the leaves present either a uniform mottled appearance, or else a curious pattern of wavy lines of darker green which follow the veins of the leaf (Plate III, fig. 2). That the virus is still active in the mature plant has been proved by the inoculation of the juice into fresh seedlings. As compared with White Burley tobacco, the Virginia variety exhibits a very strong resistance to ringspot. In all the writer's inoculation experiments, both by needle and aphis, but particularly the latter, this varietal resistance is borne out.

In addition the disease is definitely less harmful to Virginia than to White Burley. As already stated a "ringspot" Virginia plant may be normal in appearance except for isolated double rings scattered sparingly over the leaf surface. Nevertheless, this resistance is only comparative, and ringspot is easily spread from plant to plant by needle inoculation.

(b) *White Burley*. The first developments of ringspot in White Burley are somewhat similar to those in the foregoing variety; the rings, however, very rapidly lose their individuality and merge into necrotic spots and blotches (Plate II, fig. 1). Occasionally the symptoms may appear as patches, consisting of half double ring and half necrotic spot. The "spot necrosis" type of disease is decidedly characteristic of White Burley. In this variety the disease has a decidedly deleterious effect upon the health of the plant, as a rule the White Burley infected with ringspot is stunted and poorly developed. It often shows a curious one-sided

growth, and may die without reaching maturity. The different manifestations of ringspot in tobacco are shown in Plates I and II. In addition to the two types of rings, there is the spot necrosis, the lines of darker green following the veins, the so-called "clearing of the veins" often associated with the early development of the disease, and the aphid-induced mottling described in the next paragraph. In all, six series of experiments were successful in inducing ringspot in tobacco by means of needle inoculation with potato mosaic, and one unsuccessful. Six different mosaic Arran Victory plants were used as the sources of inoculum in the series during the years 1927-1928. Inoculations with juice from one potato of another variety exhibiting mosaic also produced typical ringspot.

The time elapsing before the appearance of symptoms in the inoculated tobacco plants appears to be governed largely by the temperature, and incubation periods ranged from 34 days during the winter to 12 days or less during July and August.

(b) *By aphid.*

The series of mosaic Arran Victory potato plants used in the needle inoculations were also used for the aphid transmissions, and the species employed was *Myzus persicae* Sulz. After feeding upon the mosaic foliage for a minimum period of one week the insects were colonised upon young tobacco seedlings. It is only possible to feed aphides upon these two varieties of tobacco while the plants are in the seedling stage, after this the development of sticky hairs renders aphid colonisation impossible. In all, eight series of experiments with aphid transmission of potato mosaic to White Burley and Virginia tobacco were carried out. Of these, six were successful in White Burley and two in Virginia, added proof of the greater resistance in the latter. The disease produced in the tobacco by the aphid was distinctly characteristic. It resembled in some ways the spot necrosis type produced in White Burley by needle inoculation, but yet had a distinctive character. The first manifestation consists of a "clearing of the veins." In this the veins stand out more than is normal from the rest of the leaf; this later develops into a well-marked mottle accompanied by numbers of lighter-coloured spots. So far the writer has not succeeded in producing definite concentric rings in tobacco by means of the aphid, but in one series the aphid produced the nebulous watermark type of ring. In old plants this "spot mottle" has sometimes merged into the dark green lines following the leaf veins, which also may be found in mature plants with needle-induced ringspot (Plate III, fig. 1).

The times elapsing between the date of first infection of the tobacco plant with aphid and the appearance of the symptoms varied between 44 days in January and 15 days in June. By the regular production of a disease in tobacco by means of *Myzus persicae* from mosaic potato, evidence is presented that the aphid does actually pick up the mosaic virus from the infected potato plant. The reasons for the frequent failure of the aphid to transmit the virus, once picked up, to healthy potatoes under the writer's conditions are yet to seek. It is of interest to find from the foregoing experiments that the virus of potato mosaic has apparently undergone some slight change in the body of the insect. At any rate the symptoms of the disease produced in tobacco by aphid from mosaic potato are quite distinct from the symptoms produced by needle inoculation from the same source. That this change is only slight is proved by the experiments detailed in Section 4, which show that the aphid mottle of tobacco and the true ringspot both produce identical symptoms upon healthy potato.

## SECTION 2.

Inoculation of healthy potato plants with the virus of various forms of tobacco ringspot:

### (a) *By needle.*

A large number of inoculations into healthy potato plants (varieties Arran Victory and President) were made with tobacco ringspot in all its manifestations. The needle inoculations were carried out in the same manner as those into tobacco, and scratches were made into the potato leaf-blade and petiole, and some tissue was also introduced into a stem incision. Altogether fifteen series of experiments, using about sixty plants, were made. Symptoms commenced to develop in the inoculated potato plant in periods ranging from 7 to 21 days at temperatures below 80° F. Not one of the sixty plants inoculated failed to develop disease. The disease thus produced may be compared to the original potato mosaic before its passage through the tobacco, but differing in the intensification of the mosaic mottling and above all in its enormously increased infective power. The development of this intensified or "ringspot" form of mosaic is more constant in potato than in tobacco. (For want of a better term, this intensified mosaic in the potato will be referred to as "ringspot" mosaic for convenience and not because ringspots are formed upon the potato leaf.) The first indication is the formation of well-marked spots of light green or yellow upon the youngest leaves. These spots increase in size and number, spreading over almost the entire plant with the excep-

tion of the older leaves, presenting a most brilliant mosaic mottling. The younger leaves also sometimes lose their regular outline and become crinkled and distorted. At the same time as the mosaic mottle is developing on the younger leaves, large numbers of fine necrotic spots, blackish red in colour, appear on the older leaves (Plate V, fig. 1), accompanied by a few larger lesions of the same character. These necrotic spots may also occur on the young leaves, but are more usually confined to the lower leaves. After about a week or 10 days the small necrotic spots disappear, and no new lesions are developed. The characteristic mottling, however, persists for the remainder of the life of the plant. In some cases this disease appears to have a temporarily arresting effect upon the development of the plant. Instances were observed where the potato plant remained stationary for several weeks; during that period the mosaic mottling became fixed and necrotic, the mottled areas remaining as white dead patches. After a period of 4 or 5 weeks the plant would start into active growth again, but fresh mottling of a very marked character would develop on each new leaf as formed, showing that the virus was still vigorous within the plant. In some potato plants which have been infected for 5 or 6 weeks and which have grown normally, the writer has noticed a tendency of the "ringspot" mosaic to revert, so far as the mottling goes, to the appearance of the original mosaic before its passage through the tobacco, quite apart from any suppression of symptoms by temperature. Whether the virus is actually reverting to its original condition or whether, as seems possible, the symptoms flag owing to the approaching maturity of the plant is not known at present. The writer, however, has often noticed in ordinary potato mosaic the disappearance of the symptoms with continued growth of the plant. The disease produced in the healthy potato by inoculation with tobacco ring-spot appeared the same whatever the manifestation of the tobacco disease at the time of inoculation, *i.e.* necrotic rings, nebulous rings, mottle, etc. Two experiments were also performed of inoculating healthy potato plants with the virus of tobacco ringspot which showed some increase in virulence after progressive inoculation through succeeding White Burley tobacco plants. The disease developed in the potato in both experiments in 12 days. In five out of six plants the symptoms were those characteristic of the intensified form of mosaic, but in the sixth (President) the lesions were of an unusually severe type.

*(b) By aphids.*

Although the virus of the ringspot or intensified mosaic in potato is so infectious that a needle scratch rarely fails to pass over the disease to healthy plants, it has not adapted itself proportionately to transmission by the aphid. Out of seven series of transmission experiments with the aphid *M. persicae*, involving a total of fifty plants, only two plants (Plate IV, fig. 2), with a possible third, became infected with the disease in periods of 14 days and 24 days from the date of first infection with the aphid.

## SECTION 3.

Are the varying symptoms produced in tobacco by needle inoculation with potato mosaic manifestations of the same disease?

In order to determine whether this difference in symptoms was due merely to a difference in varietal response to the virus, or whether there existed one or more diseases, the following cross-inoculations were made. Healthy Virginia plants were inoculated with the juice from White Burley tobacco plants showing the characteristic spot necrosis. In 15 days the Virginia plants showed the typical necrotic double ring. Healthy White Burley plants were inoculated with juice from Virginia showing necrotic rings. In 16 days the White Burley plants developed a typical spot necrosis. These cross-inoculations offer fair evidence that the varying symptoms are varietal presentations of the same disease. With regard to the other manifestations of ringspot in both tobaccos, such as clearing of the veins, the different mottlings, etc., it has often been found that one type will grade into another, and this together with the evidence presented in Section 2, where both forms are shown to produce identical symptoms in healthy potato plants, seems sufficient justification for supposing that there is only one disease under consideration, more especially when it is realised that all have a common source in mosaic potato. Diagnosis of virus disease from the symptoms, however, is notoriously unreliable, and the matter could probably only be settled by a study of the physical properties of the virus.

## SECTION 4.

Comparison of the symptoms produced in tobacco by aphid and needle inoculation respectively from the same mosaic potato.

Five series of needle inoculations into healthy potato plants with this aphid-induced mottle were carried out. In all, seventeen plants of Arran Victory and President were inoculated; in each case the potato plants

developed an intensified form of mosaic in no way differing, so far as the symptomatology of the disease can indicate, from the disease produced by needle inoculation into healthy potatoes of the needle-induced tobacco ringspot. Added to this is the fact that, although the aphid-induced disease in tobacco has not yet shown in the concentric ringspot form, the ringspot form and the aphid-mottle have been found to merge into similar symptoms in mature plants. For these reasons, and bearing in mind that the same mosaic potato plant was the source of infection, the writer considers that the aphid-mottle and the various forms of ringspot in tobacco are all manifestations of the same virus disease.

#### SECTION 5.

Transmission of tobacco ringspot to healthy tobacco:

##### (a) *By needle.*

Ringspot of tobacco is very easily transmissible to healthy tobacco plants by means of needle inoculation, more easily in the case of White Burley than in Virginia. Symptoms in inoculated plants develop in 7 to 14 days at temperatures above 70° F. Occasionally symptoms develop on the inoculated leaf, and rings may form along the inoculation scratches. This is often found after a series of progressive inoculations through successive plants, and is a point of difference from the "ringspot" mosaic of potato where local symptoms never occur. It often happens in needle inoculation of tobacco ringspot that some inoculations do not "take" on certain plants, about one in six failing to develop the disease. This seems to be due to some idiosyncrasy in the individual plant as conditions of inoculation are identical. The symptoms of ringspot in tobacco may develop in two ways, either on the inoculated leaf as is shown in Plate III, fig. 4, where necrotic rings form along the needle scratches, or, more usually, as a mottling on the youngest leaves of the plant, the rings developing later. In potato the "ringspot" mosaic always develops on the youngest leaves, never at the inoculation point.

##### (b) *By aphid.*

The source of infection in the first experiment was the White Burley plant illustrated in Plate I, fig. 2, which exhibited brilliant necrotic rings. This plant was colonised with the aphid *M. persicae* and, after 4 days, these were transferred to three White Burley and three Virginia plants. Nine days later the White Burleys showed the "spot mottle" disease which is typical of aphid infection of healthy tobacco plants from mosaic



potato. The Virginia plants remained healthy. In the second experiment White Burley plants, showing a very virulent form of necrotic mottling, which had been produced by progressive inoculations (see Plate III, fig. 3), were colonised with the aphid as before, and these were transferred later to healthy White Burley plants. In 12 days these White Burleys developed the mottling typical of aphid infection. It is of interest here to compare the results of a parallel series of needle inoculations from the same source, whereas the virulent form of necrosis, when transmitted by aphid, produced a mild mottling, the same when transmitted by needle produced a still more severe form of necrosis. Three points arise from these experiments: firstly they indicate that ringspot is an aphid-borne disease among tobacco plants, at least of the variety White Burley; secondly they emphasise the greater resistance of Virginia to aphid-borne ringspot; and thirdly they offer further evidence that the same virus, when aphid transmitted, produces different symptoms in the healthy plant from those produced by the virus when needle transmitted. Two possible causes may be suggested for this: either the virus has undergone some change in the body of the aphid, or else the different symptoms are induced by the different mode of inoculation to the plant.

#### SECTION 6.

Transmission of potato "ringspot" or intensified mosaic to healthy potato plants:

##### (a) *By needle.*

Seven series of experiments with needle inoculation of the intensified form of potato mosaic into healthy potatoes were carried out upon two varieties, Arran Victory and President, involving about twenty-five plants. The symptoms of the disease arising in the inoculated plants differed in no way from those produced by inoculation with tobacco ringspot on potato, the same brilliant mottling and necrotic spots being present. The disease developed in periods of 10 to 19 days, provided the temperatures were not above 80° F. The "ringspot" mosaic was just as easily passed by needle inoculation from potato to potato as from ringspot tobacco to potato.

##### (b) *By grafting.*

The "ringspot" mosaic was easily communicable from diseased to healthy potato plants by grafting. The symptoms appearing in about 9 days at temperatures below 80° F.

(c) *By aphid.*

Six series of experiments to induce the aphid *M. persicae* to transmit the "ringspot" mosaic from infected to healthy plants proved negative; both haulm and sprouted half tubers were colonised with the aphid without result. It occurred to the writer that the condition of the infected plant used as the source of infection for the aphid might be of importance. Consequently experiments were tried using as the source plants newly infected with "ringspot" mosaic and showing the symptoms very markedly. These experiments were negative also, there being no result whether the source was a diseased plant of long standing or one newly infected.

SECTION 7.

What is the effect on the virus of tobacco ringspot of progressive inoculations through successive generations of tobacco plants?

A series of progressive needle inoculations of ringspot was made in order to determine the changes, if any, induced in the virus by passage through succeeding generations of tobacco plants; three sets of experiments were performed. In the first experiment a start was made with juice from a mosaic Arran Victory potato plant, which produced concentric rings in Virginia tobacco. This ringspot was passed through five generations of tobacco plants, Virginia predominating, the times of development of the symptoms being 7 to 8 days between each set of plants. At the third set of plants, necrotic rings formed along the inoculation scratches (Plate III, fig. 4), but this did not always happen, and at the last series no perceptible increase in virulence of the virus could be detected. In the second experiment, starting with the same mosaic potato juice, the virus was passed through eight succeeding generations of tobacco plants, almost wholly of the variety White Burley. The seventh series of plants exhibited a most brilliant necrotic ringing (Plate I, fig. 3), unusual in the variety White Burley, which does not easily produce rings. In the eighth series of plants both varieties were used, and here the virus showed signs of loss of virulence and a tendency to return to the mottle and ringspot form of early infections. Furthermore, the brilliant rings did not persist throughout the life of the individual plant but, as new leaves were formed, the symptoms reverted to the characteristic spot necrosis of White Burley.

The third experiment gave somewhat different results, and was performed as follows: in the transmission experiments of potato mosaic virus to healthy tobacco, it was noticed that one series of Virginia plants

inoculated from a particular mosaic Arran Victory potato exhibited an unusually severe type of necrosis (Section 1, Exp. 4). One plant of this series was therefore selected for the third trial of successive inoculations, and juice from this plant was inoculated through a succeeding series of White Burley and Virginia plants. The first series, consisting of White Burley plants only, developed the spot necrosis type of disease in an accentuated form after 14 days. The second series of plants, White Burley and Virginia, developed in 10 and 12 days respectively an exceedingly severe type of necrosis of the veins. In the White Burleys the plants were almost killed, while the Virginias exhibited a very severe necrosis. The virus from one of these necrotic plants was then passed on to another series of White Burley and Virginia plants, which developed symptoms in 11 days. These plants showed a further increase in virulence of the virus and were practically destroyed. It is worthy of note that half-grown plants inoculated with this virus were not nearly so seriously affected as the young seedlings. That this severe disease was ringspot in some form was shown by the occurrence of isolated rings on the leaves of the Virginia before they were destroyed by the virulent necrosis. Plants infected with this virus developed new leaves which grew normally for some days until the necrotic lesions developed on them.

In this case, therefore, the plants did not grow away from the severe symptoms as had been the tendency hitherto, but succumbed to the disease. Some evidence is thus offered that continued passage of the ringspot virus through successive generations of tobacco plants does lead to an increase in the virulence of the virus, especially where the virus passes through a plant host which is favourable to it, such as the tobacco variety White Burley. As a general rule this increase in virulence is maintained in individual plants for a period only, the severe form of symptom, ring or necrosis, is exhibited only for a short time after inoculation; later new leaves appear which develop a less virulent form of symptom (Plate III, fig. 3). That this is not always the case is shown by the third experiment in this series where progressive inoculation increased the virulence of the disease until it killed the plants. It is possible that some additional factor of which the writer has no knowledge was present in this case.

In conclusion, the suggestion is made that passage of the ringspot virus through a succession of plants, favourable to its development, *does* produce an increase in virulence but usually only up to a point; when that point is reached the virus tends to return to and maintain its original level of intensity. Further experimentation is being carried out on this point.

## SECTION 8.

What is the effect on the virus of potato "ringspot" or intensified mosaic of progressive inoculations through successive generations of potato plants?

A parallel series of progressive inoculations to those carried out in the tobacco was performed to determine if any increase in virulence resulted. This experiment was only carried as far as the fourth plant, but so far as it went there were no indications of increasing virulence. Each plant developed the typical mosaic mottling and necrotic lesions.

## SECTION 9.

Inoculation of healthy tobacco plants with the virus of potato "ringspot" or intensified mosaic:

(a) *By needle.*

The virus of the intensified form of mosaic resulting in potatoes inoculated from ringspot tobacco, was returned again to healthy tobaccos. Six series of tobacco plants were inoculated from different potatoes showing the intensified mosaic. The symptoms developed in the tobacco plants in periods of 11 to 19 days. Sometimes in Virginia plants the typical rings were formed, but much more usually the first symptoms consisted of a type which might be called "clearing of the veins" (Plate II, fig. 5). This type of symptom seems especially characteristic of ringspot virus that has passed through potato. This clearing of the veins may persist for a longer or shorter time, but is generally a prelude to the development of the "mottling" manifestation of ringspot. From these experiments it would appear as if passage through potato has not materially altered the ringspot virus. It still shows a tendency to form rings upon Virginia tobacco and the mottling produced in both varieties does not differ very materially from that produced on many occasions by the virus when in the form of ordinary potato mosaic it was first transmitted to tobacco.

(b) *By aphid.*

Aphides (*M. persicae*) were colonised upon an Arran Victory potato affected with typical intensified or "ringspot" mosaic, later they were transferred to healthy White Burley tobacco plants. Symptoms of mottling developed on the tobacco in 12 days, this mottling did not differ in appearance from that produced by aphid inoculation with ordinary potato mosaic. This experiment shows that the intensified form of mosaic in potato is transmissible to tobacco by the aphid *M. persicae*.

## SECTION 10.

Needle inoculation of healthy tobacco plants with the juice of known healthy Arran Victory potatoes:

Recent work in America has suggested the possibility of healthy potatoes carrying a virus which may be toxic to other plants, and Johnson(3) has found that inoculations with juice from apparently healthy potatoes produces a disease in tobacco. Four series of inoculations with the juice of healthy potato plants into healthy tobacco were carried out. The potatoes used in this experiment were part of a stock of known healthy Arran Victory grown for three years under insect-proof conditions. No symptoms of any kind resulted in the tobacco plants thus inoculated. The writer would suggest from this that the potatoes used by Johnson, although to all appearances healthy, must have been "carrying" mosaic, that is, infected with the disease but not showing the symptoms. Carriers are known of most if not all the other potato virus diseases, and the same may be true of mosaic.

## SECTION 11.

Effect of temperature on the symptoms of ringspot:

*(a) In potato.*

There seems little doubt that temperature plays an important part in the development of this disease, both in tobacco and potato. All symptoms of the "ringspot" or intensified form of mosaic are suppressed in the potato above 80° F. (Plate V, fig. 2), and it is even difficult to infect a potato plant with the disease at or above that temperature. "Ringspot" mosaic flourishes best in the potato at about 60° to 65° F. In the glasshouse it was found that the symptoms appeared and disappeared with the fall and rise of temperature. As the details of transmission experiments of ringspot to potato will show, the times elapsing between inoculation of the potato and the appearance of the symptoms increases as the temperature rises, this being the exact reverse of what occurs in tobacco.

*(b) In tobacco.*

Below about 50° F. the tobacco plant will not flourish but remains in a stationary condition. In these circumstances, although it is possible for a plant to be infected, no symptoms of ringspot manifest themselves until the temperature rises above 65° F. The symptoms, however, of a tobacco plant once infected with ringspot do not disappear at low tem-

peratures. The disease in tobacco seems to show best between 75° to 80° F. Tobacco plants inoculated with potato mosaic on December 7th, did not manifest the true ringspot until January 20th, a total of 44 days at the low temperatures then prevailing. On the other hand, healthy tobaccos inoculated with potato mosaic on June 26th developed typical ringspot symptoms on July 8th, a total of 12 days, at a mean daily maximum temperature of 82° F. It may be seen at once from the details of the transmission experiments of potato mosaic to tobacco that the time elapsing between inoculation of the tobacco and appearance of the symptoms decreases, within limits, as the temperature rises, and the same is essentially true of the aphid transmission.

#### Filtration of the virus:

Through the kindness of Dr Henderson Smith, juice from a ringspot Virginia tobacco plant showing the typical double rings, and from an Arran Victory potato plant with "ringspot" mosaic was filtered in the following manner(2). The plants were pulped and passed first through muslin, then through tubes of sand and paper pulp, and finally through two Pasteur-Chamberland filter candles, L 1 and L 3 respectively. Some of the resulting fluid was inoculated three days later as follows: the juice from the ringspot Virginia tobacco was put into six Virginias, six White Burleys, and three healthy Arran Victory potato plants. The filtered juice from the "ringspot" Arran Victory potato was put into three Virginia tobacco, and three healthy Arran Victory potato plants. The results were as follows: the three potato plants inoculated from the ringspot Virginia developed typical "ringspot" mosaic in 11 days, while the healthy Virginias, inoculated with the same inoculum, developed the disease in 12 days. The three healthy potatoes inoculated with the filtered juice from the "ringspot" mosaic potato developed typical "ringspot" mosaic 16 days later, as did also two of the Virginia tobacco plants.

This seems conclusive evidence that the ringspot disease of tobacco is a filterable entity.

#### SECTION 12.

Inoculation of tobacco plants with virus combinations of which potato mosaic is a constituent part:

Two virus combinations only have so far been used in this series of experiments, *i.e.* streak and mosaic inoculated with the needle, and leaf-roll and mosaic inoculated by means of the aphid *M. persicae*.

The streak-mosaic combination was produced by grafting a mosaic Arran Victory with a streak-carrying Up-to-date plant. When the streak

symptoms were fully developed upon the Arran Victory, juice from this plant was inoculated by the needle into two series of White Burley tobacco. A mottling disease developed in 6 days and 14 days respectively. This mottling did not appear very different from that produced in the same kind of tobaccos by inoculation with potato mosaic only. Juice from these tobaccos was then inoculated into a series of healthy Arran Victory and President potato plants. In 9 days all the potato plants developed the typical intensified form of mosaic produced by inoculation with ringspot tobacco. There were no signs of streak in any of the potato plants, both varieties of which are exceedingly susceptible to this disease.

*Leaf-roll and mosaic.* This combination was produced by the infection of a mosaic Arran Victory with leaf-roll by means of the aphid *M. persicae* which the writer has shown to be an efficient carrier of this disease. Aphides (*M. persicae*) were colonised on this potato plant and later transferred to White Burley tobaccos. In these plants the typical aphid-mottle was produced, in no way differing from that associated with aphid inoculations from mosaic potato only. That the aphid in this case was carrying both viruses, and that the tobacco only received one has been shown by the writer in another communication, shortly to be published on the aphid transmission of leaf-roll.

From these preliminary experiments with virus combinations upon tobacco, some evidence is presented that potato mosaic only is associated with ringspot of tobacco and its various manifestations.

### SECTION 13.

Needle inoculation of plants other than tobacco or potato with the virus of tobacco ringspot:

#### (a) *Solanaceae.*

Tomato plants developed a mottling on the leaves but the writer has not yet succeeded in producing rings.

*Petunia* also showed mottling with some slight distortion of the leaves.

*Datura* inoculated with ringspot White Burley which showed very necrotic rings (Plate II, fig. 2), developed first a mottling which later turned into large necrotic lesions (Plate IV, fig. 1). True rings have not yet been produced in *Datura*, though a slight tendency to a ring-like form has been observed.

*Solanum nigrum* gave no symptoms when inoculated with tobacco ringspot.

(b) *Other plants.*

Negative results have been obtained by inoculation of ringspot virus into (a) Spinach, (b) Cabbage.

## 6. DETAILS OF EXPERIMENTS.

*Section 1 (a). Needle inoculation of tobacco with potato mosaic.*

No. of exp.	No. of plants inoculated	Date* of inoculation	Appearance of first symptoms	Incubation period days	Mean daily maximum temperature ° F.	No. of plants infected	Symptoms
1	3 White Burley 3 Virginia	Dec. 7	Jan. 10	34	57	{3 W.B. 2 V.	Mottling followed by double rings on Virginia and spot necrosis on White Burley.
2	3 White Burley 3 Virginia	Feb. 23	Mar. 15	21	72	{3 W.B. 3 V.	Rings on Virginia; spot necrosis on White Burley.
3	6 White Burley	Mar. 3	Mar. 20	17	74	4 W.B.	Spot necrosis.
4	6 Virginia	Mar. 8	Mar. 22	14	75	6 V.	Rings in most cases; some mottling. Severe necrosis in one plant.
5	3 White Burley 3 Virginia	June 26	July 8	12	82	{3 W.B. 3 V.	Spot necrosis on White Burley concentric rings and nebulous rings on Virginia.
6	3 White Burley	June 27	July 10	13	82	3 W.B.	Spot necrosis. Half spot, half ring in one plant.

*Section 1 (b). Aphis (M. persicae) inoculation of tobacco with potato mosaic.*

1	3 White Burley 3 Virginia	Dec. 7	Jan. 20	44	62	3 W.B.	Characteristic "spot mottle" on White Burley; Virginia healthy.
2	6 White Burley 6 Virginia	Feb. 22	Mar. 23	30	73	{5 W.B. 2 V.	Typical mottle later turning to spot necrosis.
3	3 White Burley	Apr. 24	May 11	17	82	3 W.B.	Typical aphid mottle.
4	3 White Burley 3 Virginia	June 15	June 30	15	87	3 W.B.	Typical aphid mottle.
5	3 White Burley	June 10	June 25	15	90	2 W.B.	Typical aphid mottle.
6	6 White Burley	June 28	July 15	17	84	5 W.B.	Typical mottle with nebulous "watermark" type of ring on one plant.

\* In Section I (b) date of inoculation = date of colonisation of plant with the aphid.



## Section 2 (a). Needle inoculation of potato with tobacco ringspot.

No. of exp.	No. of plants inoculated	Date of inoculation	Appearance of first symptoms	Incubation period days	Mean daily maximum temperature ° F.	No. of plants infected	Remarks
1	6 Arran Victory	Feb. 21	{ Feb. 27 to Mar. 3 }	6-11	68	6	—
2	3 Arran Victory	Mar. 5	Mar. 12	7	71	3	—
3	2 Arran Victory	Mar. 7	{ Mar. 18 and Mar. 21 }	11-14*	69	4	—
	2 President						
4	3 Arran Victory (inoculated with filtered virus)	Apr. 4	Apr. 16	12	77	3	—
5	6 President	Apr. 13	Apr. 26	13	76	6	—
6	6 President	May 2	May 11	9	75	6	—
7	3 President	May 7	May 16	9	77	3	—
8	3 President	June 4	{ June 25 and July 2 }	21 and 28	85 86	3	—
9	3 President	May 15	May 26	11	79	3	—
10	3 President	June 2	June 20	18	84	3	—
11	3 Arran Victory	June 2	June 18	16	80	3	—
12	3 Arran Victory	June 11	June 27	16	80	3	—
13	3 Arran Victory	June 14	June 30	16	80	3	—
14	4 President	June 16	June 30	14	80	4	—
15	{ 3 half tubers Arran Victory (inoculated in sprouts) }	May 26	June 25	40	86	3	—

\* 14 days = older Arran Victory; 11 days = young President.

## Section 2 (b). Inoculation of potato with tobacco ringspot by aphid.

1	3 President	Mar. 13	Mar. 27	14	68	1	Young seedlings used.
2	5 President (2 of above exp. re-infected)	Mar. 16	—	—	68	Negative	—
3	3 President	Apr. 16	May 10	24	75	1	Seedling plants used.
4	3 President	Apr. 16	Apr. 30	14	72	1	Slight mottling only.
5	3 President	Apr. 20	—	—	75	Negative	In this experiment a newly infected plant was used as source of infection.
6	24 sprouted Arran Victory half tubers with half tuber controls	Apr. 22	—	—	75	Negative	—
7	3 Arran Victory	May 3	—	—	75	Negative	—

In experiment No. 7 three young Arran Victory plants were colonised with aphid from a tobacco plant which had itself been infected with the mottle disease by aphid from mosaic potato. Juice from this tobacco plant when inoculated by needle into healthy potato produced the typical "ringspot" or intensified mosaic. The aphid, however, failed to carry the disease back to potato, although it brought it readily enough to the tobacco.

Section 3. See page 14.

*Section 4. Needle inoculation into healthy potato of the aphid-produced mottle in tobacco.*

No. of exp.	No. of plants inoculated	Date of inoculation	Appearance of first symptoms	Incubation period days	Mean daily maximum temperature ° F.	No. of plants infected	Remarks
1	3 President	Apr. 12	—	—	—	—	These three plants remained apparently healthy.
2	4 President	May 14	June 4	21	82	4	The inoculum used in this experiment was the same as in No. 1, but was passed through an additional generation of tobacco.
3	4 President	May 14	May 26	12	76	4	—
4	3 Arran Victory	May 31	June 7	8	80	3	Symptoms were masked by temperature on June 7 and disappeared until July 2.
5	3 President	June 20	July 10	20	80	3	—

*Section 5 (a). Needle transmission of tobacco ringspot to tobacco.*

No. of exp.	No. of plants inoculated	Date of inoculation	Appearance of first symptoms	Incubation period days	Mean daily maximum temperature ° F.	No. of plants infected	Remarks
1	3 White Burley	Jan. 30	Feb. 14	15	63	3	—
2	3 White Burley	Feb. 15	Feb. 27	12	78	3	—
3	6 Virginias	Feb. 15	Feb. 22	7	78	5	—
4	3 Virginias	June 21	July 1	10	86	2	—
5	3 Virginias	Feb. 17	Mar. 3	15	64	3	—
6	3 White Burley	Feb. 16	Mar. 3	14	64	3	—

*Section 5 (b). Aphis transmission of tobacco ringspot to tobacco.*

3 White Burley	}	July 28	Aug. 7	10	83	3 W.B. Virginia is apparently negative	The aphides were colonised on tobacco with pronounced "rings," but the disease produced in the new tobaccos, was the typical "spot mottle."
3 Virginia							
2 4 White Burley		Aug. 15	Aug. 27	12	80	4 W.B.	—

In experiment 2 the aphid were colonised on White Burley tobacco which was affected with the very severe type of necrosis; the four infected plants, however, showed only the typical aphid mottle.

*Section 6 (a). Needle transmission of potato "ringspot" or intensified mosaic to healthy potato plants.*

No. of exp.	No. of plants inoculated	Date of inoculation	Appearance of first symptoms	Incubation period days	Mean daily maximum temperature ° F.	No. of plants infected	Remarks
1	3 Arran Victory } 3 President }	Mar. 30	Apr. 10	11	78	6	Source of infection was a "ringspot" Arran Victory.
2	3 half-grown Arran Victory } 3 very young Arran Victory }	Mar. 30	Apr. 16 to Apr. 18 Apr. 11	17-19 12	75 78	6	Illustrates the shorter incubation period in younger plants.
3	3 Arran Victory	Apr. 2	Apr. 18	16	78	3	Inoculum used was filtered (see Section 11).
4	3 President (seedlings)	May 25	June 4	10	78	3	—
5	3 President (seedlings)	June 6	July 2	34	85	3	Symptoms masked by temperature.
6	1 Arran Victory } 1 President }	June 25	July 10	15	78	2	—

*Section 6 (b). Transmission by grafting of potato "ringspot" mosaic to healthy potato.*

1	Arran Victory grafted with diseased scion of Arran Victory	May 26	June 10	14	78	1	—
2	Arran Victory grafted with diseased President	May 26	July 1	35	82	1	Symptoms masked by temperature.

*Section 6 (c). Aphis transmission of potato "ringspot" mosaic to healthy potato.*

Six series of experiments on both haulm and sprouting half tuber. All these were negative.

*Section 7. What is the effect, on the virus of tobacco ringspot, of progressive inoculations through successive generations of tobacco plants?*

No. of exp.	Generations of tobacco	Date of inoculation	Appearance of first symptoms	Incubation period days	Remarks
1	(a) Virginia	Feb. 15	Feb. 22	7	In (d) typical necrotic rings formed along the inoculation scratches (Plate III, fig. 4). Exp. No. 1 finished at (e); there was no perceptible increase in virulence.
	(b) White Burley	Feb. 16	Feb. 24	8	
	(c) Virginia	Mar. 23	Apr. 1	8	
	(d) Virginia	May 2	May 10	8	
	(e) Virginia	May 23	May 31	8	
2	(a) Virginia	Feb. 1	Feb. 8	7	Both Exps. 1 and 2 started with the same virus. In Exp. 2, at the 5th generation (e), very brilliant necrotic rings developed (Plate I, fig. 2). In (f) the symptoms have reverted to characteristic spot necrosis.
	(b) Virginia	Feb. 15	Feb. 22	8	
	(c) White Burley	Feb. 16	Feb. 24	8	
	(d) White Burley	Mar. 23	Apr. 2	9	
	(e) White Burley	June 25	July 4	9	
	(f) White Burley	July 30	Aug. 10	11	
3	(a) Virginia	Mar. 8	Mar. 24	16	The source of inoculation in Exp. 3 was a Virginia plant showing unusually necrotic spotting, once from mosaic potato. Necrosis increased in (b). All plants show a very severe form of necrosis (see Plate III, fig. 3). Virulence still further increased. Plants practically killed.
	(b) White Burley	June 4	June 27	23	
	(c) White Burley	July 26	Aug. 5	10	
	Virginia	July 26	Aug. 7	12	
	(d) White Burley	Aug. 9	Aug. 20	11	

*Section 8. See page 19.*

*Section 9 (a). Needle inoculation of healthy tobacco plants with the virus of potato "ringspot" or intensified mosaic.*

No. of exp.	No. of plants inoculated	Date of inoculation	Appearance of first symptoms	Incubation period days	No. of plants infected	Remarks
1	6 Virginia	Mar. 7	Mar. 21	14	5	Some rings, and mottling.
2	6 Virginia	Mar. 16	Mar. 23	17	6	Majority of plants show "clearing of the veins." Some rings.
3	3 Virginia	Mar. 30	Apr. 10	11	3	Symptoms typical of ring-spot.
4	3 Virginia	Apr. 2	Apr. 21	19	3	Inoculum used was filtered juice. Symptoms typical rings but faint. Virus appeared slightly attenuated by filtration.
5	3 White Burley	June 25	July 7	12	3	"Clearing of the veins" followed by mottling. Symptoms died out in one case.
6	3 White Burley 3 Virginia	July 30	Aug. 8	9	3 W.B. 1 Virginia	"Clearing of the veins."

*Section 9 (b). Aphis inoculation of healthy tobacco plants with the virus of potato "ringspot" or intensified mosaic.*

1	3 White Burley	Aug. 19	Aug. 31	12	3	A mottling disease produced which did not differ in appearance from that produced by aphis inoculation with ordinary potato mosaic.
---	----------------	---------	---------	----	---	---

## 7. DISCUSSION.

The outstanding experience of the writer in his work with the insect transmission of potato mosaic has been the difficulty of obtaining an insect which would carry the virus with any degree of regularity. This difficulty does not seem to be shared with other potato virus workers on the continent and in the United States (Elze<sup>(1)</sup>, Schultz and Folsom<sup>(6)</sup>). The aphid *Myzus persicae*, so largely used in this work, is the insect to which the writer has paid greatest attention in his virus studies because it is the one which has given nearly all the positive results obtained. Not only is it the chief, if not the sole, vector of potato leaf-roll in this country, but it is concerned, practically all over the world, with the dissemination of plant viruses of many kinds. This apparent affinity for virus diseases, emphasised by its omnivorous habits and world-wide distribution, renders *Myzus persicae* an insect of the gravest importance.

The non-success of the insect transmission of potato mosaic led to the experimentation with the tobacco plant described in this paper. The utilisation of the tobacco as an indicator has demonstrated one important fact, *i.e.* that the aphid *M. persicae* does actually pick up the virus when it feeds upon a mosaic potato plant. The demonstration of this fact renders the reasons for its failure to infect the potato, under the writer's experimental conditions, difficult to fathom. As the foregoing experiments indicate, the aphid infects the tobacco from the mosaic potato with great ease and will also carry the virus on from tobacco to tobacco, but immediately the infective aphid is brought into contact with the potato, the positive infections cease. Inoculation of tobacco with the virus of a potato mosaic has given rise to several further points of importance. It has demonstrated that a potato mosaic and tobacco "ringspot" are apparently caused by the same virus; this immediately suggests the potato fields as a source of tobacco ringspot in America, with the aphid *M. persicae* as the probable connecting link. That the same virus in another plant host should react in so different and striking a manner is of considerable interest. Moreover, the symptoms of the disease in tobacco, when in the ringspot form, are particularly striking. What is it that causes a disease, the usual symptoms of which in one plant are a mild mottling, to develop in another plant host the peculiar double concentric rings shown in Plate I, figs. 1 and 2, and what is the significance of rings? These rings are symmetrical and very clearly cut, giving the appearance of being stamped on artificially. It is probable that the virus of potato mosaic undergoes some change in the tissues of the tobacco

plant. This change does not appear to be of a fundamental nature, because ringspot returned to potato reappears in a mosaic form. It is true that the symptoms are intensified and the infective power enhanced, nevertheless it is still a disorder of the mosaic type. There are other points of difference in the behaviour of the virus in the two plant hosts—in tobacco the symptoms show best at 80° F., while in potato the symptoms are masked at that temperature and positive inoculation is difficult if not impossible. As the potato plant grows well at 65° F., and the tobacco at 80° F., and as it is at these temperatures that the symptoms show best in the respective plants, optimum conditions for the plant host are apparently optimum conditions for the virus. In tobacco local symptoms often appear at the point of inoculation, but never in potato where first symptoms always develop on the young leaves. A parallel case of one virus being concerned in two different diseases is found in the work of Kunkel(4) who shows that Aster Yellows is caused by the same virus as that producing White Heart of lettuce.

A further point of interest lies in the results obtained by respective inoculations of tobacco by needle and aphid. Although the same mosaic Arran Victory potato was used as the source of infection in both cases the resulting symptoms were entirely different, each one characteristic of its method of inoculation. The needle inoculation produces the rings or typical spot necrosis, while aphid infection produces symptoms of a marked type consisting of a yellowish spotting accompanied by a mottling of darker green<sup>1</sup>. Also, when the severe form of disease illustrated in Plate III, fig. 3, was inoculated into fresh plants by means of the needle, death resulted in the inoculated plant, but when transmitted by the aphid the typical aphid mottle only resulted. It may be suggested that the virus has undergone a change in the body of the aphid, but if so the change is presumably not great, as, when returned to healthy potato by needle, the disease produced is symptomatically identical with that produced by true ringspot. It may be that this disparity in symptoms arising between aphid and needle inoculation of tobacco is due to the difference in the mode of inoculation. The writer has shown(7) that *M. persicae* consistently taps the phloem of its plant host, so that presumably the virus is introduced to the phloem of the tobacco plant by the aphid with greater consistency than by a needle scratch on the leaf blade. This difference may be sufficient to account for the divergent symptoms. Comparative inoculations of the two varieties of tobacco, White Burley

<sup>1</sup> The lines of darker green shown in Plate III, fig. 1, are very characteristic of aphid-borne infection of tobacco plants.

and Virginia, have demonstrated that there exists between them a considerable difference of susceptibility to the ringspot virus. This difference in susceptibility is particularly strong in the case of aphid-borne infections. It is as easy to infect White Burley tobacco with the spot mottle disease by means of the aphid as it is difficult to infect Virginia tobacco with the same disease by means of the aphid. This varietal susceptibility is also shown though to a less extent in needle inoculations. Were it not for the fact that both the varieties of potato used, *i.e.* Arran Victory and President, are exceedingly susceptible to the disease, difference in varietal susceptibility to mosaic might explain the writer's non-success with aphid transmission of this disease to potato.

It is a commonplace of plant viruses that active growth and movement in the host is an essential for infection and subsequent development of the virus. This is emphasised in the present studies where young potato plants inoculated with the virus of ringspot developed symptoms in 12 days, as compared with 17 to 19 days in older plants under similar conditions. Further, tobacco plants in a pot-bound, and consequently stationary, condition were very difficult to infect with ringspot. Symptoms in tobacco plants already infected, tended to disappear when the plant became pot-bound and to reappear when the plant re-started into active growth. It has been shown that ringspot in tobacco, and its counterpart in potato are filterable entities; in this the writer's results differ from those of Priode<sup>(5)</sup>, who found that the ringspot disease of tobacco would not pass a grade "N" Berkfeldt filter.

It is evident that much work yet remains to be done both concerning insect transmission of potato mosaic and the affinities of this disease with tobacco ringspot. Although at the moment some of the results obtained may appear incompatible, it is hoped that, as knowledge of a difficult subject grows, these apparent anomalies may disappear.

#### 8. SUMMARY.

(1) Some positive evidence of the transmission of potato mosaic by the aphid *Myzus persicae* Sulz. is given.

(2) Attempts to induce a number of other potato-feeding insects to transmit the disease proved negative.

(3) It has not been found possible to infect potato plants with mosaic by means of inoculation with the body juices, or salivary glands, of insects which have been bred upon mosaic potato plants.

(4) The virus of a potato mosaic inoculated into healthy tobacco plants by means of the needle produces an infectious disease—ringspot—

of which the most characteristic symptom is the formation of necrotic concentric rings with a central spot. The symptoms of this disease differ in the two varieties of tobacco used—White Burley and Virginia—and the susceptibility of the former is greater.

(5) The virus of a potato mosaic transmitted to healthy tobacco by means of the aphid *M. persicae* produces a characteristic spot and mottle disease which is considered to be substantially the same disease as the needle-produced ringspot.

(6) Ringspot in its various manifestations, when inoculated back to healthy potato by needle or aphid, reproduces in the potato the original mosaic with the symptoms intensified, and the infective power greatly enhanced. Although the aphid readily carries the virus of potato mosaic to tobacco, it transmits the resulting disease back to potato only with very great difficulty.

(7) This intensified mosaic can be spread from potato to potato by needle scratch with the greatest ease, but not by the aphid *Myzus persicae* Sulz.

(8) The spot and mottle disease produced in tobacco by aphid from mosaic potato, when inoculated into healthy potato plants by the needle, produces the same intensified form of mosaic as does the needle-induced ringspot in tobacco, when returned to healthy potato.

(9) Tobacco ringspot can be spread from tobacco to tobacco by needle or aphid. When transmitted by aphid the symptoms differ from those produced by the needle.

(10) It is proved that the aphid *Myzus persicae* picks up the virus from mosaic potato with great regularity, but has so far usually failed under the writer's experimental conditions to infect healthy potato plants with the disease.

(11) The virus of tobacco ringspot is shown to increase in virulence by progressive inoculation through successive generations of tobacco plants. The increase is greatest after passage through a plant which is highly favourable to the development of the virus, such as the susceptible variety of tobacco, White Burley. This increase in virulence in some cases reaches only to a certain point, after that it tends to revert to its original intensity, but in others the virulence reached an intensity sufficient to kill the plant. As a rule increased virulence of the virus in a given plant does not persist throughout the life of that plant.

(12) Temperatures above 80° F. mask the symptoms of the intensified mosaic in potato, but not of ringspot in tobacco. The respective symptoms show best in potato at 60° to 65° F. and in tobacco at 80° F. Needle



inoculation on tobacco often produces local symptoms at the point of inoculation, but never on the potato where first symptoms appear on the young leaves.

(13) Juice from a potato plant infected with the intensified mosaic, and from a ringspot tobacco plant, was filtered through two Pasteur-Chamberland filter candles, L 1 and L 3. The resulting filtrate originating from each plant infected healthy potato and tobacco plants with their respective diseases. This shows that the ringspot virus, and its counterpart in potato, are filter-passing entities.

(14) Juice from known healthy potato plants when inoculated into healthy tobacco plants produced no symptoms.

(15) The virus of tobacco ringspot was inoculated into various plants with the following results:

- (a) Tomato. Mottling on the leaves, rings not yet produced.
- (b) *Petunia*. Mottling on the leaves with some distortion.
- (c) *Datura* sp. Mottling accompanied by large necrotic areas.
- (d) *Solanum nigrum*. No symptoms.
- (e) Spinach. No symptoms.
- (f) Cabbage. No symptoms.

Only two plants each inoculated in (d), (e), (f).

#### 9. REFERENCES.

- (1) ELZE, D. L. (1927). The Dissemination of Virus Diseases of the Potato by Insects. *Inst. voor Phytopath. Lab. voor Mycol. en Aardappelonderzoek. Meded.* 32.
- (2) HENDERSON SMITH, J. (1928). Experiments with a Mosaic-Disease of Tomato. *Ann. App. Biol.* xv, No. 2, May.
- (3) JOHNSON, JAMES (1925). Transmission of Viruses from Apparently Healthy Potatoes. *Agricult. Exp. Station Univ. of Wisconsin Bull.* 63, Sept.
- (4) KUNKEL, L. O. (1926). Studies on Aster Yellows. *Amer. Journ. Bot.* XIII, No. 10, Dec.
- (5) PRIODE, C. N. (1928). Further Studies in the Ringspot Disease of Tobacco. *Amer. Journ. Bot.* xv, Jan.
- (6) SCHULTZ, E. S. and FOLSOM, D. (1925). Infection and Dissemination Experiments with Degeneration Diseases of Potatoes. Observations in 1923. *Journ. Agric. Research*, xxx, No. 6, March.
- (7) SMITH, KENNETH M. (1926). A Comparative Study of the Feeding Methods of Certain Hemiptera and of the Resulting Effects upon the Plant Tissue, with Special Reference to the Potato Plant. *Ann. App. Biol.* XIII, No. 1, February.
- (8) — (1927). Observations on the Insect Carriers of Mosaic Disease of the Potato. *Ann. App. Biol.* xiv, No. 1, February.



Fig. 1



Fig. 2.



Fig. 3.



Fig. 4.





Fig. 1



Fig. 2



Fig. 3



Fig. 4



Fig. 5



Fig. 6





Fig. 1.



Fig. 2.



Fig. 3.



Fig. 4.





Fig. 1.



Fig. 2.



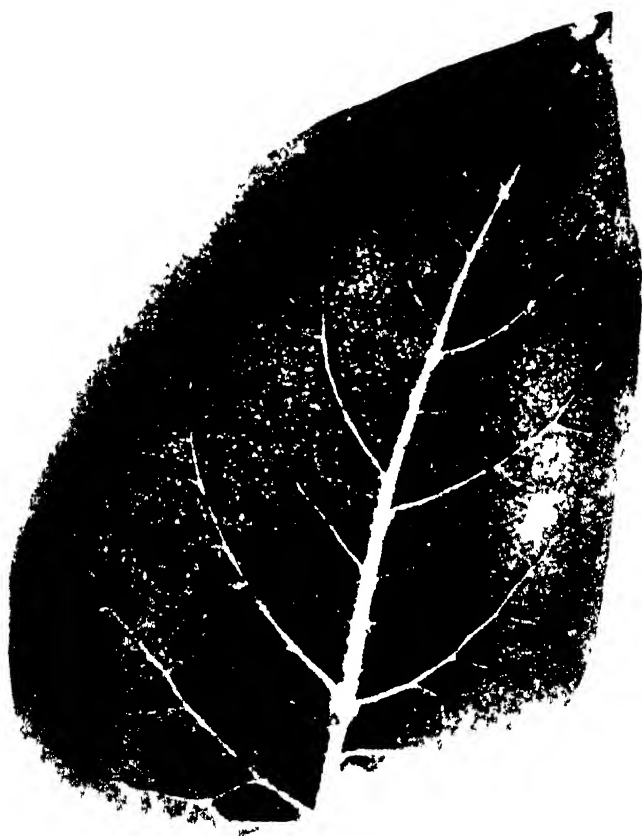
Fig. 3.



Fig. 4.









## EXPLANATION OF PLATES I—V.

## PLATE I.

- Fig. 1. Typical ringspot symptoms on Virginia tobacco. These symptoms are the result of a direct inoculation from mosaic Arran Victory potato. Note the double rings in the lower right-hand corner. Average diameter of rings about 5 mm.
- Fig. 2. Rings produced in White Burley tobacco, after passing the virus through five successive generations of tobacco. Size of leaf, 9 in., rings varied in diameter from 5 to 8 mm.
- Fig. 3. Hieroglyphic type of marking produced in White Burley, usually after a number of progressive inoculations.
- Fig. 4. Nebulous or "watermark" type of ring. This occurs in both varieties, and is the only type of ring yet produced by aphid inoculation from mosaic potato.

## PLATE II.

- Fig. 1. Young White Burley tobacco plant infected with ringspot, the rings are rapidly turning into the spot necrosis characteristic of the disease in this variety.
- Fig. 2. Older White Burley plant, the leaves may be seen covered with spots and blotches, some of which still retain a ring-like form.
- Fig. 3. Spot necrosis symptoms developing in young White Burley.
- Fig. 4. Young White Burley inoculated with the same virus as in Fig. 3. Here the symptoms are tending towards ring formation.
- Fig. 5. "Clearing of the Veins." A common early symptom of ringspot in both varieties of tobacco.
- Fig. 6. Spot necrosis as it often appears in an old White Burley plant.

## PLATE III.

- Fig. 1. Leaf of White Burley plant showing symptoms produced by aphid inoculation from mosaic potato. At the time of photographing, this plant had been infected for some weeks. The typical spotting produced by the aphid may be seen at the apex of the leaf. Length of leaf, 7 in.
- Fig. 2. Leaf of mature White Burley plant inoculated by needle from mosaic potato, for comparison with Fig. 2. Note the same picking out of the veins with darker green is found in both.
- Fig. 3. Young Virginia plant inoculated with the virus which has been passed through a number of White Burley plants. Note the severe necrosis in two leaves. The new leaves show signs of growing away from the severe form of the disease, these leaves later developed necrosis less severe than in the older leaves.
- Fig. 4. Rings forming along the inoculation scratches on a leaf of Virginia.

## PLATE IV.

- Fig. 1. Leaf of *Datura* plant inoculated with tobacco ringspot. Note the very severe necrotic lesions developing on the leaf. Size of leaf, 3½ in.
- Fig. 2. One of the few successful transmissions to potato by means of the aphid *M. persicae*. "Ringspot" or intensified mosaic in a healthy President potato plant produced by aphid from ringspot Virginia tobacco.
- Fig. 3. "Ringspot" or intensified mosaic on President produced by needle inoculation from ringspot tobacco. The light and dark areas represent the characteristic mottling of this disease.
- Fig. 4. "Ringspot" or intensified mosaic in Arran Victory produced by needle inoculation from ringspot tobacco. Photographed by transmitted light.

## PLATE V.

- Fig. 1. Leaf of Arran Victory potato, showing the large and small necrotic lesions occurring in "ringspot" mosaic. The small lesions appear in the photograph as numbers of black specks along the outer uppermost leaf margin.
- Fig. 2. Stem on the left cut from potato plant showing the intensified form of mosaic, that on the right masked by temperature.

Photographs by C. W. Williamson.

(Received August 24th, 1928.)

# ON TWO CASES OF RECOVERY FROM A MOSAIC DISEASE OF TOMATO PLANTS, *LYCOPERSICON ESCULENTUM*

BY LEN VERWOERD, M.Sc. AGRIC. (STELL.).

(Department of Phytopathology, College of Agriculture, University of Stellenbosch, Stellenbosch, C.P., Union of South Africa.)

## 1. INTRODUCTION.

DURING recent years much has been added to our knowledge concerning the mosaic diseases which affect plants belonging to different families and orders. Although many obscure facts have found elucidation, there are still many perplexing questions which remain unsolved.

Most workers on mosaic diseases are agreed that if a plant has once become infected with mosaic there exists little hope for true recovery. Reports of recovery from mosaic diseases are naturally sceptically viewed by critical students of this problem. If such recovery is eventually proved, then it would appear to be of an exceedingly rare occurrence. The disappearance of mosaic symptoms have been observed by various workers. Allard<sup>(1)</sup> noticed that plants of *Nicotiana glauca* soon lost their mottled appearance, but he demonstrated the presence of the infective principle in such plants and found it highly virulent. This observation was later confirmed by Dickson<sup>(6)</sup> in his experiments on mosaic. Melhus<sup>(10)</sup> records that the eggplant, *Solanum melongena*, lost its mosaic characters after the plants had passed the seedling stage. Cases have been reported<sup>(3,9,11)</sup> of plants, which had previously shown mosaic infection, producing new shoots free from this disease. It is also known that *Physalis alkekengi*<sup>(11)</sup> and *Physalis francheti*<sup>(7)</sup> never show symptoms of mosaic mottling, though they may contain the active principle which causes mosaic symptoms when injected into tobacco plants.

None of the cases above referred to can be considered as indicating true recovery from mosaic. As far as I am aware, only one case<sup>(4)</sup> has thus far been reported which would tend to indicate the possibility of recovery from mosaic. This record is, however, based on negative results in only one inoculation trial.

The disappearance of symptoms of mosaic disease, which Lodewyks<sup>(8)</sup>, Chapman<sup>(5)</sup>, and Dickson<sup>(6)</sup> reports to take place when mosaic plants are

grown in blue light, was subsequently shown by Dickson<sup>(6)</sup> to be one of masking and not true recovery. This he demonstrated by infecting healthy plants which had been exposed to blue light and also by removing some of these plants to sunlight when they again developed mosaic symptoms.

## 2. EXPERIMENTAL.

During an epidemic spell of a mosaic disease of tomatoes in the months of December 1927 to February 1928 the writer had many thousand tomato plants under continual observation on the experimental farms of the University.

Three hundred tomato plants of the varieties Bonnie Best, Livingstone's Coreless and Norduke Wilt Resistant, which were infected with mosaic, were cut back at the time of flowering. A large percentage of these failed to produce new shoots. It is probable that the majority of the plants which did not again sprout were those most severely affected. A number of these plants which failed to sprout were dug up and their root systems examined. In all these cases the root development was of a normal character. In all cases where shoots developed the mosaic condition was reproduced in the new growth. The results of this experiment are recorded in Table I.

Table I.

Variety	No. of plants				
	With distinct mosaic symptoms	Cut back	Developing mosaic shoots	Not developing mosaic shoots	Not developing shoots
Bonnie Best	100	100	76	Nil	24
Livingstone's Coreless	100	100	60	Nil	40
Norduke Wilt Resistant	100	100	83	Nil	17
	300	300	219	Nil	81

Fifty tomato plants which showed no symptoms of mosaic and which were standing amongst those recorded in Table I were also cut back. These plants were not isolated and were liable to mosaic from the diseased plants by natural means. With three exceptions these plants developed healthy shoots showing no visible symptoms to indicate the presence of mosaic disease. The new growth of the three plants, which developed mosaic symptoms, could possibly have become infected by natural means from the diseased plants amongst which they stood. The results of this experiment are recorded in Table II.

## 36 *Recovery from a Mosaic Disease of Tomato Plants*

Table II.

Variety	No. of plants				
	Ap- parently mosaic free	Cut back	De- veloping mosaic shoots	Not de- veloping mosaic shoots	Not develop- ing shoots
Bonnie Best	25	25	1	22	2
Norduke Wilt Resistant	25	25	2	23	0
	50	50	3	45*	2

\* Thirty of the 45 plants which did not develop mosaic were subsequently used as a source for inoculum to test whether the disease was not perhaps latent or masked in these plants.

Allard(1) found that the virus of mosaic disease may be present in a highly virulent condition and yet not produce visible symptoms of mosaic on the host. This fact was subsequently confirmed by Dickson (6).

With the object of testing whether this was not perhaps also the case with the plants mentioned in Table II as being free from mosaic, the juice of thirty of these plants was extracted separately and each was injected into ten tomato plants raised under controlled conditions. Twelve weeks after inoculation the plants were growing vigorously and showed no signs of the disease on either foliage or fruit. At the same time mosaic-free tomato plants were inoculated by the same method with juice from diseased plants and these developed mosaic disease. Whilst visiting some tomato fields at Kleinvele, Clanwilliam, in December 1927 a number of cuttings showing mosaic symptoms were taken from a badly infected plant and planted at Stellenbosch. An equal number of cuttings, taken from a mosaic-free plant, were planted out at the same time. Table III shows the result of this experiment and, as indicated there, the plants raised from two of the mosaic diseased cuttings showed no symptoms of mosaic.

Table III.

Date of plant- ing	No. of cuttings planted	Mosaic or mosaic- free	No. of cuttings rooting	Subsequent growth		Date of final observa- tion
				Mosaic	Mosaic-free	
Dec. 1927	24	Mosaic	6	4	2	10. iv. 28
	24	Mosaic-free	20	0	20	

In order to determine whether the infective principle was not perhaps latent or masked in the two healthy plants raised from diseased cuttings, the following infection experiments were made.

Twenty-five tomato plants (*vide* Series A, Table IV) were inoculated by means of the hypodermic syringe method with juice from the two

healthy and apparent mosaic-free plants mentioned in Table III. When three weeks had elapsed and no mosaic symptoms developed on the inoculated plants, two further series of twenty tomato plants were inoculated. The plants in the one series were inoculated by puncturing the host tissue through drops of juice (*vide* Series B, Table IV) and twenty tomato plants (*vide* Series C, Table V) by inserting small fragments of host tissue. The results of these inoculation experiments are given in Table IV.

Table IV.

Series	Date of inoculation	No. of plants	Variety	Method	Source of inoculum	Reaction	Remarks	Final date of observation
A	30. i. 28	25	Bonnie Best	Hypodermic syringe	The apparent mosaic-free plants	-	—	—
		25	Bonnie Best	Hypodermic syringe	Mosaic plant	+	Control	—
B	21. ii. 28	20	Bonnie Best	Punctures through drops of juice	The apparent mosaic-free plants	-	—	15. iv. 28
		20	Bonnie Best	Punctures through drops of juice	Mosaic plant	+	Control	—
C	21. ii. 28	20	Bonnie Best	Insertion of fragments of tissue	The apparent mosaic-free plants	-	—	—
		20	Bonnie Best	Insertion of fragments of tissue	Mosaic plant	+	Control	—

As may be inferred from the above table, no plants showed any symptoms of mosaic. The two mosaic-free plants raised from diseased cuttings eventually produced normal healthy fruit, free from mosaic.

In the course of the above experiments it appeared desirable to plant cuttings from the two mosaic-free plants raised from diseased cuttings, in order to ascertain whether the plants raised from them would remain free from mosaic. The season was by this time already well advanced, but nevertheless ten cuttings were planted from one of these plants. Only four of these cuttings rooted and the plants raised did not grow as vigorously as those planted from cuttings earlier in the season. Five weeks after planting the cuttings, the plants which developed showed no evidence of mosaic. Three tomato plants were inoculated with juice from these plants but without developing any signs of mosaic. From the experimental evidence cited, the author is led to conclude that the two mosaic-free tomato plants raised from diseased cuttings represent true recoveries from this disease.



## 3. TECHNIQUE.

It seems desirable to give a brief account of the methods used in the experiments recorded.

*Material.* The tomato plants used in testing the transmissibility of the virus or infective principle were grown away from the pot-house where the inoculation experiments were carried out. The plants were raised from carefully selected seed in tins and the necessary precautions taken to exclude possible insect vectors. As soon as the young plants could be transplanted they were potted out in 6-inch pots. From amongst these plants vigorous ones, 5 to 10 in. high, were selected for inoculation experiments. This type of plant was selected because in some preliminary work on the interspecific transmission of mosaic diseases in Solanaceous plants such plants gave better results.

*Inoculum.* Except where otherwise stated, the juice of plants which served as inoculation material was obtained by macerating in a mortar the leaves and tips of young shoots of the plants. In this way a pulpy mass was obtained, which was diluted with a little distilled water and then filtered through filter-paper.

To obtain inoculation material containing the active principle of mosaic, plants were selected which showed well-developed symptoms of this disease.

*Inoculation.* Experiments in which plants were injected with an inoculum obtained from mosaic-free plants were controlled by injecting the juice of mosaic diseased plants into the same number of plants and *vice versa*.

In inoculating the plants, great care was taken to touch the plants as little as possible. Before introducing the inoculum the hands were thoroughly washed with soap and water. In each case, the plant to be inoculated was held between the thumb and index finger by means of a bit of cotton-wool, which was renewed for each plant. The juice was injected into the stem 2 in. above soil level and also at the base of a young leaf at the tip of the plant.

For injection a hypodermic syringe was generally used. In the case of the experiments mentioned in Table IV inoculations were also made: (a) by puncturing the host with a sterile needle through drops of juice obtained either from plants definitely known to be free from mosaic or from the two plants raised from mosaic-infected cuttings and which showed no visible sign of mosaic; (b) by inserting fragments of tissue obtained either from plants definitely known to be free from mosaic or

from the two plants raised from mosaic-infected cuttings and which showed no visible sign of mosaic, into the host by means of a curved arrowhead needle.

#### 4. SUMMARY.

Experiments are recorded which indicate that from a number of tomato plants raised from mosaic-diseased cuttings two plants were free from mosaic disease.

These two plants showed no symptoms of mosaic disease and neither was the active principle of the disease present in the juice obtained from these plants.

#### REFERENCES.

- (1) ALLARD, H. A. (1917). Further studies on the mosaic of tobacco. *Journ. Agric. Res.* x, 615-631.
- (2) BEYERINCK, M. W. (1898). *Verhand. kon. Akad. van Wetenskappe, Amst.* xi, 6/5.
- (3) BRANDES, E. W. (1919). The mosaic disease of sugar cane and other grasses. *U.S. Dept. of Agric. Bull.* 829, 1-26.
- (4) BRIERLEY, W. B. (1915-16). A case of recovery from mosaic disease of tomato. *Ann. App. Biol.* ii, 236-266.
- (5) CHAPMAN, G. H. (1916). The effect of coloured light on mosaic of tobacco. *Science*, N.S. XLIV, 537-538.
- (6) DICKSON, B. T. (1922). Studies concerning mosaic disease. *McDonald College, Quebec, Tech. Bull.* ii, 1-125.
- (7) ELMER, O. H. (1925). Transmissibility and Pathological effects of the mosaic disease. *Iowa College of Agric. Amer. Res. Bull.* 82.
- (8) LODEWYKS, J. A. (1910). Zur Mosaikkrankheit des Tabaks. *Rec. Trav. Bot. Neerl.* vii, 107-129.
- (9) LYON, H. S. (1921). Three major cane diseases: mosaic, serch and Fiji disease. *Bull. Hawaiian Sug. Plant. Exp. Stn*, iii, pt. 1, 1-43.
- (10) MELHUS, I. E. (1919). Notes on mosaic symptoms of Irish potatoes. *Phytopath.* vii, 71.
- (11) NISHAMURA, M. (1918). A carrier of the mosaic disease. *Bull. Torrey Bot. Club*, XLV, 219-233.
- (12) WOOD, F. A. (1902). Observations on the mosaic disease of tobacco. *U.S. Dept. of Agric. Bur. Pl. Ind. Bull.* 18.

(Received June 28th, 1928.)

## STUDIES OF WOOD-DESTROYING FUNGI

I. *POLYPORUS HISPIDUS* (FRIES)

BY F. J. NUTMAN, A.R.C.S., B.Sc.

(Department of Scientific and Industrial Research Forest Products  
Research Laboratory.)

(With Plates VI-VIII and 2 Text-figures.)

## CONTENTS.

	PAGE
INTRODUCTION . . . . .	40
CULTURAL CHARACTERISTICS . . . . .	42
Production of fruit bodies in culture . . . . .	45
Growth on inoculated wood blocks . . . . .	45
Growth of hyphae . . . . .	46
THE PARASITISM OF <i>POLYPORUS HISPIDUS</i> . . . . .	50
THE PENETRATION OF THE CELL WALL . . . . .	51
Penetration by the tip of a young hypha . . . . .	53
Penetration by means of a peg-like outgrowth . . . . .	53
THE ENZYMES OF <i>POLYPORUS HISPIDUS</i> . . . . .	54
SUMMARY . . . . .	63
REFERENCES . . . . .	63
EXPLANATION OF PLATES . . . . .	64

## INTRODUCTION.

*POLYPORUS HISPIDUS* Fr. is one of the chief fungi attacking ash in this country. Rea<sup>(15)</sup> lists it as "common" and it is certainly of quite frequent occurrence, especially as it is one of those fungi which remain comparatively unnoticed until a search is made. This is due to the fact that the fruit body grows rather high up on the tree, and is of somewhat ephemeral nature.

It occurs generally on ash and apple. Rea gives walnut as an additional host, and I have frequently found small fructifications on elm, which is given as a host by Schroeter. Other hosts are mulberry (Prilleaux<sup>(14)</sup>), plane (Schroeter), and oak (Murrill<sup>(13)</sup>).

It is interesting to note that in America *P. hispidus* occurs on the Black Ash (*Fraxinus nigra*) almost exclusively, and where mixed stands of *F. nigra* and *F. excelsior* occur it attacks the former, never being found

on white ash, any decay of the latter being caused by *Fomes fraxinophilus* exclusively. In England *P. hispidus* occurs on both black and white ash, and I have collected sporophores from both. Owing, however, to the greater abundance of white ash, the fungus is chiefly important as causing a decay of this tree.

The decay produced in white ash is very similar to that described by Baxter<sup>(1)</sup>, the decayed wood being conspicuously lighter in colour and softer than sound wood, and cracking along the growth rings when dried. In nearly all cases examined, the source of infection was the stub of a dead branch, the rot spreading up and down the tree for a considerable distance. The sporophores usually appear at the point of infection, but in some cases no connection with an injury could be seen. Although the non-occurrence of any obvious wound or dead stub cannot be taken to imply the absence of infection through a wound, it is possible that the fungus gained its entry into the tree through some minor injury, which exposed the living tissues for a time long enough for them to be attacked. It will be shown later that *P. hispidus* is capable of attacking young sapwood, though it is normally a heart-rotting fungus.

Trees attacked by this fungus show little sign of its presence; at any rate trees bearing sporophores and showing little evidence of weakening are common. Later on, however, they begin to die back, and eventually death ensues.

One of the most important points of interest about the fungus is its possible connection with that condition of ash known as "brashness<sup>1</sup>." Apparently sound ash will often be found to break under a strain much below the normal, the fracture being of a peculiar brittle nature. In many cases this "brashness" is obviously due to the poor quality of the timber used, since ash which is slow grown and which contains an unduly large proportion of the weak spring wood will naturally be weak. In some instances, however, the "brash" fracture occurs in wood which is anatomically sound; that is to say, wood that is indistinguishable from that of high strength by the microscopic examination. A number of such examples have been examined, and in every instance the presence of a fungus has been demonstrated in the wood, the hyphae being very sparsely scattered, though they are evenly distributed. As yet no cultures have been obtained from such samples, the inference being that the fungus has died out in the seasoned timber. As sporophores have

<sup>1</sup> The term "brashness" arose in America, and is now in common use in the timber trade of both this country and the U.S.A. In this paper its use will be restricted to that condition of timber, however caused, which produces a peculiar "carroty," short, fracture.

never been seen by the writer on fallen branches of ash or on dead trees, it is possible that the conditions for growth of this fungus are such that it dies out subsequent to felling. As far as I am aware, no record exists of the fungus being found on dead wood.

Baxter states that mycelial wefts are found in trees decayed by this fungus up to a distance of 6 ft. from the last stage of visible decay or discoloration, and that the wood in this region is infected with mycelium. The fact that mycelium can be demonstrated in wood at a considerable distance from the visible decay has been well established by Hubert<sup>(10)</sup>, and observations made on decayed ash trees confirm his results. Hence a proportion of the timber from any infected tree, while *apparently* sound, may yet contain a considerable amount of fungal mycelium.

Unfortunately, in the present state of our knowledge we do not know the precise effect of a fungus on wood during the early stages of decay, but from general observation it seems probable that a considerable proportion of the wood from a tree which is slightly decayed should be suspect. While in some circumstances the decrease in strength may not be of any great practical importance, yet at other times, for example in aeroplane construction and in the manufacture of tennis rackets, strength is the very factor for which the wood used is selected. It is well known that in the trade, ash, particularly English-grown ash, is highly valued for its great toughness and elasticity.

The fungus most commonly found on ash is undoubtedly *P. hispidus*. *Fomes fraxineus* may sometimes be met with, but it is not nearly so frequent in occurrence, being listed as "uncommon" by Rea. If, therefore, any definite relation between "brashness" and fungal infection should be found to exist, the fungus chiefly concerned will probably prove to be *P. hispidus* with *F. fraxineus* occupying a subordinate position.

This paper is the result of a cultural study of this fungus, and of its enzymes, preliminary to a more general investigation of the problem of "brashness" in timber.

#### CULTURAL CHARACTERISTICS.

*Polyporus hispidus* grows readily, though slowly, on all the ordinary culture media, solid or liquid, though less rapidly on synthetic media such as Richard's. For this work ordinary 2 per cent. malt agar was found to be most satisfactory, and was used throughout. The fungus at 25° C. covers an ordinary 4-inch petri dish of this medium in about one month.

On agar the mycelium forms a thick mat on the surface of the medium, the stout hyphae forming a plush-like growth overlying a dark brown

skin-like membrane formed in contact with the agar. The mycelium is bright antimony yellow—lighter in the younger portions and shading into buckthorn brown in the older parts; all the colours used in the description of the fungus are according to Ridgeway. No traces of fructification have been observed on solid media although the usual expedients of varying the temperature, altering the humidity, changing the concentration of food materials, etc., have been tried. Baxter obtained similar negative results on attempting to induce fruiting.

In liquid media the fungus makes very good growth, especially on turnip extract. This extract was prepared by autoclaving cubes of turnip without the addition of water and expressing the juice, which, when filtered and sterilised, proved to be an ideal medium for the fungus, especially when large quantities of mycelium were required.

On this the young mycelium is mostly sub-aerial, forming a dense woolly mat of upright stout hyphae floating on the surface of the liquid. These are colourless or else a very pale yellow. In fact, on any medium where the growth of the fungus is sufficiently rapid, the coloration is never produced until staling has set in and the growth has been somewhat retarded. This also appears to be true for the majority of wood-destroying fungi, some of the more rapidly growing of which do not assume the characteristic coloration until after a considerable lapse of time. It almost appears as though some substance produced in staling is responsible for the coloration of the fungus.

On turnip extract the coloration begins to develop after about a week, starting in the centre and spreading rapidly all over the colony until the whole is a very beautiful pale barium yellow. After this stage growth slows down, the colony becomes very much more compact, and a considerable development of submerged mycelium takes place. At the same time the colour becomes much more vivid, and deeper in tone (antimony yellow), while patches of buckthorn brown appear on conical or hemispherical protuberances arising from the level mycelial mat. These often become hispid, and sometimes approach Dresden brown in colour. They were noted by Baxter, who mentions their frequent occurrence and their hispid character. In no case do they give rise to fruit bodies, as the single fructification which was produced in culture arose very rapidly from a younger perfectly level mycelial mat.

Eventually the fungal colony becomes a widespreading and gelatinous mass of submerged mycelium, bearing on its upper surface a dense felted mat of aerial hyphae, which frequently exudes dark sticky drops of a very astringent taste. The colouring matter is almost confined to the aerial

hyphae which are very uniform in size. They are of a bright yellow colour under the microscope, the colour being mostly in the cell wall.

On examining the younger stages of the submerged mycelium it was found that there was a pronounced tendency for the hyphae to branch very freely, generally dichotomously, and for these branches to become highly vacuolate. They then swell very considerably at the vacuoles, especially at the tip of each branch; in this way little clumps of denser appearance begin to occur in the mycelial mass. These clumps appear

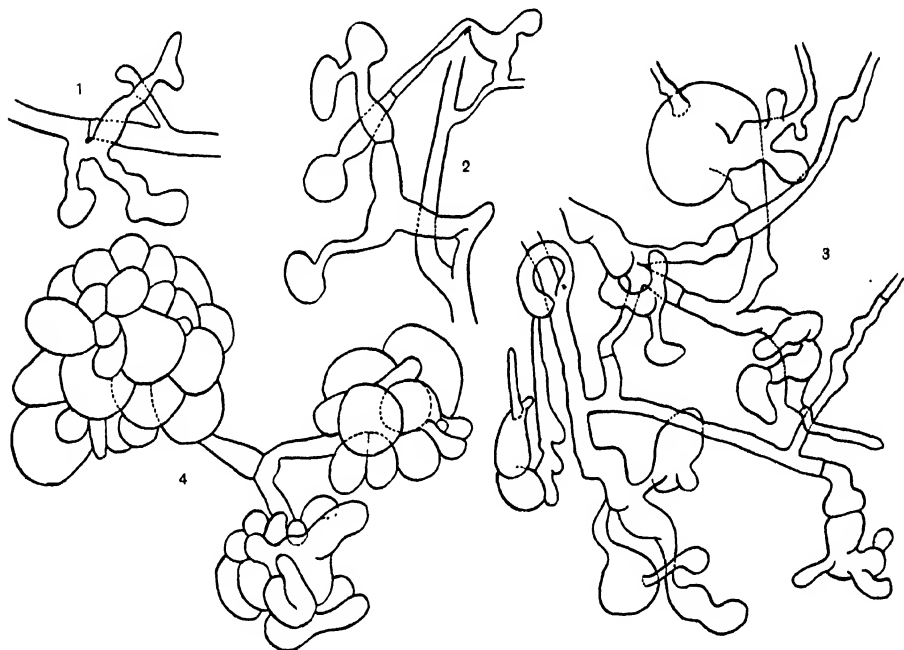


Fig. 1. Formation of plectenchyma by mycelium of *Polyporus hispidus* in liquid medium.

with some regularity, scattered throughout the felt of hyphae, and, when the mass is examined under a low power, look like clumps of large yeast cells. They spread and get denser, eventually joining up and forming a mass of vesicles which, pressing closer together as the swelling increases, form a kind of plectenchyma. Soon the appearance of hyphae is entirely lost, the only thing to be seen being a mass of small bladders resembling bunches of grapes in surface view.

It is noteworthy that no clamp connections have been found in culture, either on solid or on liquid media.

*Production of fruit bodies in culture.*

As has already been stated, the production of fruit bodies of *P. hispidus* has, in general, been absent, no expedient succeeding in inducing fruiting. However, one liquid culture suddenly produced a large irregularly hemispherical protuberance, studded with dark brown drops, which looked as though they were being excreted from a pore surface. The protuberance, too, was distinctly hispid in patches, and its general appearance was not in the least like the smaller ones referred to previously. It also differed in the rate of growth, since on Saturday midday the colony was perfectly normal, and on Monday morning the protuberance had reached the dimensions given below. After being photographed the mass was removed from the Erlenmeyer flask in which it was grown. It had become so tough that it was necessary to break the glass in order to remove the fructification. The fruit body was about 4 in. by 3 in. in extent, and 2 in. thick, the pore surface being about 3 in. by 2½ in.

On examination it was found that a definitely organised pore surface was present, with large pores averaging 0.7 mm. in diameter and about 6 mm. deep. However, there was no hymenium lining the tubes, or at any rate no well-organised one, while no spores could be found. Apparently the fruit body has just entered on its final stage of development, and was removed from the flask too soon.

The culture in question was one of many put up to obtain a large quantity of mycelium for an investigation of the enzymes, and had been kept for most of the time in an incubator at 25° C., though it had occasionally been removed and kept at room temperature for some days owing to lack of incubator room.

Westerdijk<sup>(19)</sup> states that fluctuations of temperature often induce fruiting in the higher Basidiomycetes. This explanation does not seem available here, since all the flasks in this particular batch had been subjected to the same temperature variations; also, deliberate alterations in temperature have produced no sign of fruiting.

*Growth on inoculated wood blocks.*

In order to trace the development of the hyphae in the wood of ash, small blocks were placed in a flask, autoclaved with the addition of water, inoculated with *P. hispidus* and incubated at 25° C. Some external growth was made, enough to show that all the blocks were being attacked, but not enough to be very obvious; most of the mycelial development was in the substance of the blocks.



At the end of four months samples were removed and sectioned on a Reichert microtome. As they were fully imbibed with water they sectioned readily enough without demineralisation, when a very sharp knife (Jung) was used. Sections  $9\mu$  to  $10\mu$  were easily obtained, and these were stained with safranin and picro-aniline-blue. This method of staining fungal hyphae in wood is due to Mr K. St G. Cartwright, of the Forest Products Research Laboratory and will shortly be described by him. The protoplasm of the fungus, and to a less extent the fungal cell wall, took up the blue stain, forming a brilliant contrast with the red lignified walls of the wood.

The blocks were  $1.5\text{ cm.} \times 2\text{ cm.} \times 3\text{ cm.}$  and in all those sectioned hyphae were present in the centre of the blocks. As this gave a rate of penetration much greater than that recorded by Baxter, another experiment was set up to gain more accurate information.

#### *Growth of hyphae.*

The greatest development of the hyphae at this stage of attack was in the medullary rays, nearly all the cells of which contained hyphae, branching freely but never forming a thick weft in the cells such as is formed by some species of fungi, *e.g.* *Fomes ribis*. The hyphae in the rays were very granular and dense, being full of protoplasm and obviously well nourished. They varied greatly in diameter, some being quite thick ( $3\mu$  to  $4.5\mu$ ), while the majority were about  $2\mu$ . Baxter gives  $7\mu$  as the largest hypha that he has observed. Hardly less numerous were the hyphae in the xylem parenchyma. Here the diameter was much more uniform, being on the average  $1.5\mu$ . The majority of the hyphae were of this size, though some were very fine and hyaline. No clamp connections could be observed.

At this stage of decay there was very little development of the fungus in the fibres, nor, apparently, in the vessels, as no hyphae were seen in the latter even on close examination of many sections. Later it will be shown that the absence of mycelium in the vessels is only apparent, and that in reality the vessels are attacked very early in the history of decay. The process of sectioning, however, tears the hyphae from the lumina of the vessels, suggesting that the hyphae avoids the vessels. Apart from this it appears that the fungus makes its greatest development in the medullary rays and in the xylem parenchyma, owing possibly to the more easily available food supply. In the blocks the typical decay was not produced, though a slight discoloration was seen at the periphery. No marked alteration of the staining reactions of the cell-wall was

produced, and there was apparently little effect on the wood. The general impression from examination of the sections and of the wood in bulk was that the fungus was living at the expense of the organic and other debris in the cells, and had only just begun to break down the cell-wall.

After decay had progressed for six months the distribution of the hyphae had altered very considerably. The vessels were choked with a thick weft of mycelium, though this was generally lost in the process of sectioning. The medullary rays and the xylem parenchyma were full of hyphae, much resembling those in the wood decayed for four months, but rather more abundant. The fibres by this time were vigorously attacked, any section showing many hyphae ramifying in the cells. These hyphae are quite large in diameter, full of protoplasm, and apparently active and well nourished. They bored freely in the walls, without reference to the position of any pits. It is peculiar that in the early stages of decay penetration should be almost entirely by means of the pits, while later on it should be by "bore-holes," obviously the result of enzyme activity, while pits, in general, are ignored as a means of passage from one cell to another.

An interesting feature of the bore-holes is that they are frequently formed in rows down one cell-wall by a single hypha, which winds through the wall and back again in very much the same way that a thread does in cloth. In fairly advanced decay the damage done to the cell-wall merely by bore-holes appears to be considerable, without taking into account any general weakening of the tissues which is presumably produced by the enzymes of the fungus acting more or less evenly all over the walls.

In no case were any clamp connections, medallion hyphae, or "buckles" observed in wood decayed by *P. hispidus*.

*P. hispidus* does not usually form "zone-lines" in wood, though Baxter records that in some instances brown lines are formed during decay. In the blocks decayed for six months dark lines were formed in the more decayed portions. These, under the microscope, can be seen to be due to a dark brown material filling the cavities of the cells. In suitably thin or bleached sections this material can be seen to consist of solid masses of old hyphae, large in diameter ( $7\mu$  to  $8\mu$ ), and thick-walled; they have lost their protoplasmic contents, and their walls have turned a deep brown. These dead hyphae are not confined to the cells in the "zone-line," but can be found mingled with the normal hyphae in the cells near that line. With advancing age, the normal hyphae appear to

pass over into the thick-walled type, or perhaps when there is no further nutriment in the wood.

An attempt was made to get a more accurate idea of the rate of penetration of hyphae through wood. For this purpose blocks, 5 cm.  $\times$  1 cm.  $\times$  1 cm., were cut from sound wood in such a way that the long axes were

1. Longitudinal;
2. Radial in the tree.

One dozen of each kind were put up. They were coated with sealing-wax all over, with the exception of the smaller ends, and then re-coated with colloidin. As sterilisation by autoclaving was impracticable, because of the certainty of the wood being altered by the treatment, and also because of the effect on the coating, the blocks were sterilised at 60° C. for 12 hours in a saturated atmosphere. They were then kept for one month in a saturated atmosphere in order to become uniformly imbibed, and then inoculated at the small ends with mycelium of *P. hispidus*.

After six months the blocks were removed and the sealing-wax removed. The decay had spread down the blocks with some rapidity, the wood nearest the point of inoculation being bleached to a pale yellow colour, and being obviously softer and more spongy than the unaltered wood at the distal end of the block, or the slightly attacked wood in the intermediate portions. This lighter portion of the wood extended 7 mm. on the average from the point of inoculation in those blocks in which the path of the fungus was across the growth rings, and to a slightly greater distance in the others, though in these it was not at all sharply delimited. Small pockets of the yellowish rot could be traced to a distance of 2 cm. from the point of inoculation.

The surfaces of the blocks were trimmed off very carefully in a microtome, great care being taken to ensure the surface being smooth and plane, and the blocks mounted whole on a slide. They were then examined under a 16 mm. objective and a Beck ring-illuminator. By this means hyphae could be seen in the vessels as an open, brilliantly illuminated network, standing out with great vividness against the black background of the vessels. Mycelium could also be seen in the fibres and sometimes in the rays. The visibility of mycelium in the rays is of rare occurrence, not because of any lack of hyphae, but because there is generally a cell-wall between the mycelium and the surface of the section.

By this means an estimate of the distance to which the fungus had penetrated could be made, especially as the zone containing the fungus

in the vessels was very sharply delimited from the unattacked wood, on both the transverse and the longitudinal faces of the blocks.

The distance traversed by the fungus in a radial direction, *i.e.* across the growth rings, was 3.2 cm., no block differing more than 1 mm. from the average. As wood is such a heterogeneous substance the unexpected uniformity of these results is of interest, although every precaution was taken to equalise the conditions in the various blocks. Baxter obtained a longitudinal rate of penetration of 1 mm./month, but in his case the material was young ash sprouts, so that the two results are not comparable. Unfortunately the fungus had penetrated completely through the blocks in a longitudinal direction, so that no definite data could be obtained. The results are, therefore:

Penetration across growth rings	... 5 mm./month.
Penetration parallel to the grain	... 7 mm./month, at least.

In order to prepare wood for observation by means of the ring illuminator it is to be emphasised that the surface must be rendered perfectly plane and smooth by a heavy razor, mere smoothing by a knife, plane, or scalpel not being sufficient. The open ends of the vessels and fibres must be cut cleanly across without distortion, and debris must not be allowed to fall into them. These conditions are admittedly difficult to realise, especially with some woods, but the results repay the labour expended, as it is possible to get a very accurate idea of what the fungus is like in the wood, in a way that is impossible by the examination of sections, which of course should be used as well. I have found that the easiest way of preparing the blocks is to clamp them securely in a sliding microtome, and then to take shavings from the face to be examined, using a very sharp, very heavy razor, set at an extreme angle to give a long drawing stroke. In this way a face can be prepared which is suitable for observation by reflected light.

Unfortunately it is almost impossible to obtain a successful photomicrograph of this, as the hyphae are generally some little way down in the vessels, and if they are in focus the wood is not, and *vice versa*. Also, owing to the lack of focal depth of an objective, it is generally impossible to focus the whole of the surface of the wood. When working, however, this is of no moment, as one automatically moves the fine adjustment and so obtains a composite image of the surface of the block.

In the decay of ash by *P. hispidus* the mycelium ramifies to a considerable extent in the vessels, which appear to be the paths of the fungal advance in the longitudinal direction, as the medullary rays are in the

radial. The vessels are large and when sections are cut it is rare for any hyphae to remain in them. They are either torn out by the microtome knife, or are washed away during the staining process, unless some method of embedding is used. The results are then generally unsatisfactory from other points of view, to say nothing of the inconvenience and the length of time involved. It is, therefore, easy to reach the conclusion that the hyphae are avoiding the vessels for other parts of the wood, whereas the exact reverse is the case. Under the ring illuminator the hyphae can readily be seen in the vessels, and a good general idea of the rot obtained. The magnification obtainable is not great, but the definition is very good, and the full resolving power of the lens is utilised. Owing to the great advantage of viewing a block of wood with the mycelium *in situ*, it is urged that some form of ring illumination should always be used in the description and investigation of decay in wood, at any rate in woods which have large pores.

#### THE PARASITISM OF *POLYPORUS HISPIDUS*.

It is naturally of considerable importance to determine whether *P. hispidus* is an obligate saprophyte, or whether it can attack cells which are still living. If the latter is the case the tree can be attacked through minor injuries, while if the former the attack must be through branch stubs or similar defects which expose the dead heart-wood of the tree.

Baxter succeeded in infecting freshly cut black ash sprouts, containing only sap wood, with the mycelium of the fungus, finding that it attacked the medullary ray cells readily, as well as the wood elements. He also succeeded in carrying out successful inoculations of young trees, and in tracing the mycelium some little distance into the wood.

Young ash trees, 10 years old, at Oxford, were inoculated in June 1927 with the mycelium of *P. hispidus* as follows: a T-shaped slit was cut in the bark with a sharp scalpel, and the flaps of bark pulled back. Actively growing mycelium was then placed on the wound, and the flaps pressed firmly in position. The wound was then covered with viscous paper bound tightly in position with wool, or with grafting wax. After three weeks some of the trees were removed and sectioned. The mycelium could be seen pressed closely in contact with the wood, and longitudinal sections showed the presence of young hyphae sparingly scattered in the zone immediately behind the point of inoculation. Most of the hyphae were in the medullary rays, a few being in the parenchyma of the wood. These were small ( $1.5\mu$ ), densely granular, and apparently actively growing.

Three months after inoculation other trees were removed and sectioned in a similar manner. The wound had completely healed by this time, and the original inoculum could still be seen included in the young wood. Hyphae were readily found in the medullary rays, xylem parenchyma, and vessels, immediately behind the point of inoculation. They were larger, on the whole, than those seen in July, and were obviously well nourished. They had not spread far through the wood, the greatest distance noted being 2 mm., but were quite numerous. The general impression given was that the fungus, having gained a foothold in the young wood, was flourishing there in a small area, and was invading fresh tissues slowly.

From these results, and from those of Baxter, it appears certain that though *P. hispidus* is normally a saprophyte, in that it attacks the heart-wood, yet this saprophytism is facultative, since it can, and no doubt does, attack living cells.

#### THE PENETRATION OF THE CELL-WALL.

In the blocks subjected to the action of *P. hispidus* for four months, penetration was almost entirely by means of the pits, with which the cells of the medullary rays and the xylem parenchyma abound. Penetration of the cell-wall in a ray cell has not been observed, and only occasionally in a cell of the xylem parenchyma. This is true, not only of the blocks decayed for four months, but also of those rotted for longer periods. In other words, penetration has never been observed other than by pits in either

1. A medullary ray cell.
2. A xylem parenchyma cell (except in the wall bordering on the fibres).
3. A vessel.

This is interesting with regard to a statement by Hubert, to the effect that wood-destroying fungi generally exhibit a complete indifference to the pits, often passing through the wall very near them. While this is, no doubt, true for some fungi, it is not always so, since in this case the fungus appears to prefer the pits as a passage way from one cell to another, and yet it is capable of causing a very considerable decay of the wood.

In all, 27 sections of the four months' rotted wood were examined, and in these penetration other than by pits was only noted three times. In none of these could the method of penetration be seen clearly, though

it appeared to conform to that seen in blocks at a more advanced stage of decay.

In the blocks rotted for six months, under what were probably better conditions for the growth of the fungus, penetration could be seen very frequently, and all stages could readily be observed. In general it is of

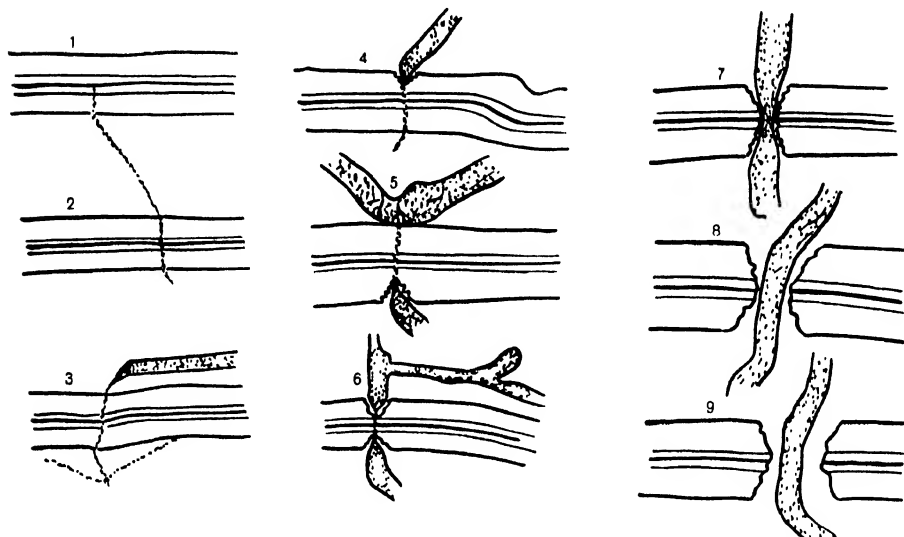


Fig. 2. Penetration of the cell-walls of ash by hyphae of *Polyporus hispidus*.

1. Fine hypha half through cell-wall.
2. Fine hypha completely through cell-wall.
3. Hypha thickening up at one end of the bore-hole.
4. Erosion beginning at one end of the bore-hole.
5. Erosion in progress at one end of the bore-hole. Hypha thickened on both sides of the cell-wall.
6. Erosion in progress at both ends of the bore-hole.
7. Bore-hole much enlarged. Hypha passing through with but slight constriction.
8. Bore-hole still further enlarged. Unstricted hypha passing through bore-hole very slightly larger than itself.
9. Final stage. Unstricted hypha passing through bore-hole much larger than itself.

two distinct types, one being common, while the other is much more rare. These are:

1. Penetration by means of the tip of a young hypha, which subsequently thickens and erodes the cell-wall.

2. Penetration by means of a peg sent out from an older and thicker hypha.

*Penetration by the tip of a young hypha.*

This appears to be the normal mode of penetration, and all stages may readily be seen in suitably stained sections. For this work the safranin-aniline-blue combination was found to be very useful, due to the brilliant contrast obtainable between the hypha and the wall which it is penetrating.

The first stage seen is when a young hypha, so fine that it is merely a line of small protoplasmic granules under a  $\frac{1}{12}$ th objective, comes in contact with a wall. It bores straight through, taking the usual shortest path, *i.e.* perpendicular to the cell-wall, with no change of diameter at all. The penetration here must be enzymatic, since it is hardly conceivable that so fine a thread, with no appressorium, could penetrate a thick lignified cell-wall by mechanical means. It is unusual and interesting that in several cases observed (figured in No. 3 and to a less extent in No. 4) the hypha turns aside from the normal path. It was at first thought that this was due to some distortion of the cell-wall caused by sectioning, but more detailed observations showed that this was not the case, since other hyphae penetrating the same cell-wall nearby were not deformed in a similar manner.

After penetration, the young hypha thickens, until it has reached approximately its mature size. The portion in the wall undergoes no change, remaining too small to be measured. At this stage the hypha is about  $1.5\mu$  in diameter, the two parts being joined by the very fine thread in the cell-wall. After this an erosion takes place round *one* end of the thin thread in the wall, followed soon after by a similar erosion at the opposite end. As this increases, two cone-shaped openings are formed in the wall with their axes along the thin fungal thread. As the cones increase in size the hypha swells up, the swelling of the hypha and the enlargement of the cones going on simultaneously, until the apices of the cones meet and pass. This produces a large bore-hole, with an unconstricted hypha passing through. By this time the outer opening of the bore-hole is much larger than the hypha. Erosion proceeds still further, however, the final stage being reached when the hypha lies in a hole the minimum diameter of which is three times that of itself, the maximum being five times.

*Penetration by means of a peg-like outgrowth.*

This is of quite rare occurrence, but may sometimes be noted in wood which has been considerably decayed. It differs from the previous type in that it takes place when an older and thickened hypha comes to lie



against a cell-wall. From the hypha a peg is put out, of approximately the same diameter as the tip of the normal type, which penetrates as before. Presumably the thickening up of this peg goes on in the same manner as that described for the previous type, but it has not been observed.

A good, though brief, review of the literature of cell-wall penetration, and an historical summary, is given by Hubert, dealing more especially with *Trametes pini* as a type of fungus whose enzymes are secreted solely by the tips of the hyphae, the bore-holes consequently never enlarging. Some of the fungi which exhibit penetration resembling that of *P. hispidus* are:

Fungus	Observer
<i>Polyporus Fulvus</i>	Hartig
<i>P. nigricans</i>	Lindroth
<i>P. betulinus</i>	Lindroth
<i>P. laevigatus</i>	Lindroth
<i>Lentinus lepideus</i>	Buller
<i>Fomes igniarius</i>	Hubert
<i>F. ribis</i>	Nutman
<i>Trametes robinophila</i>	Hubert
<i>Stereum hirsutum</i>	Nutman

#### THE ENZYMES OF *POLYPORUS HISPIDUS*.

In spite of the great importance of the higher fungi in the decay of wood, surprisingly little work has been done on their enzymes. Seeing that these enzymes are the armoury of the fungus, it behoves us to know as much as possible about them. Zeller(20) gives a brief summary of the work done on these enzymes in his paper on those of *Lenzites saepiaria*.

All work on fungal enzymes, if it is to be at all controlled, necessitates the preparation of an enzyme suspension from the fungus concerned. The means of obtaining such suspensions are often by no means free from objections. Some workers, such as Bourquelot(3), Herissey(4), and Buller(7), have expressed the juice from the sporophores of the fungus, since the sporophore affords a ready means of obtaining a large quantity of pure mycelium of the fungus under investigation. This juice has been used either directly, as an enzyme suspension, or has been treated with alcohol to precipitate the enzymes which have been re-suspended in water.

This can be objected to on two grounds: Firstly, because the mycelium in question is generally old, and, as Brown(6) has shown, the enzymes are naturally concentrated in the tips of the young actively growing hyphae. In the case where the sporophore is young and actively growing this

objection is not valid, though the second one would apply. The second objection is that the sporophore is a fruit body, and the enzymes present are not necessarily an indication of the full complement in the young vegetative mycelium. This has been well demonstrated by Zeller, in an investigation of the enzymes of the mycelium and sporophore of *Lenzites*, where he found that many enzymes, notably invertase, diastase, tannase, and cellulase are abundant in the mycelium and are scanty or absent in the sporophore. In some instances, such as the oxidases, the converse may be true.

The point is that the mycelium of the fungus may be divided into two physiologically distinct units, the first being purely vegetative and metabolic, equipped with the enzymes necessary for its particular type of metabolism, while the second is the mycelium building up the fruit body, which is not necessarily equipped for the same physiological processes as is the vegetative part of the plant. Hence, in order to get any idea of how the fungus employs enzymes in its particular mode of life, it is necessary to use the vegetative mycelium as a source of enzymes, since many such enzymes might be absent in the sporophore and would be missed if a sporophoral extract was used.

Zeller used an extract from sawdust on which the mycelium had been cultivated for a period of seven months. In such a case much of the mycelium would be old and relatively poor in enzymes. He also used an extract from the dried sporophores.

In this work on *P. hispidus* endeavour was made to grow the fungus as rapidly as possible, so that the extract could be made from young mycelium containing a large proportion of enzymes. Since the plant does not form spores in artificial culture, the method evolved by Brown for *Botrytis cinerea* could not be used and a modification of it was adopted. The mycelium was grown in large Erlenmeyer flasks in turnip extract, which was found to be most favourable for rapid growth. The flasks were inoculated with six or seven pieces of mycelium, in order to provide several centres for growth. All the cultures were incubated at 25° C. In about one month a large quantity of mycelium was produced. This was removed, washed for some time in running water, dried as thoroughly as possible between sheets of blotting paper, and the process completed *in vacuo* over recently fused calcium chloride. The dried mycelium was then ground to powder in a mortar, and kept for future use. To make the enzyme extract 10 gm. of this powder, containing about 5 gm. of mycelium and about 5 gm. of sand, was extracted with 100 c.c. of distilled water for 24 hours. The aqueous extract was filtered, and toluene was

added as an antiseptic. This will be referred to as enzyme extract No. 1.

In some cases the enzymes were precipitated from this by the action of three volumes of 95 per cent. alcohol. The flocculent precipitate was caught on a filter-paper, washed with alcohol, and the suspension reformed by allowing the paper to stand in distilled water for a few hours, with occasional gentle agitation. This enzyme suspension was often used at the same time as No. 1, and in every instance gave identical results.

Another extract was prepared from the fresh mycelium. After washing it was immediately transferred to a mortar, where it was ground up with sand as rapidly as possible. The pasty mass was then extracted with water and filtered, toluene being added to the filtrate as an antiseptic. It will be referred to as enzyme extract No. 2. In all instances where Nos. 1 and 2 were used at the same time the action was identical qualitatively, and more or less the same quantitatively, except with the catalases, where much greater effervescence was given with No. 2.

These enzyme suspensions, judging by results, are much more active than those used by other workers on the enzymes of the Basidiomycetes. For example, the guaiacum reaction for oxidase, which took two hours to develop with Zeller's extract, took place in 4 minutes with extract No. 1, and in  $3\frac{1}{2}$  minutes with extract No. 2. This might appear to be due to some excess of oxidase in *P. hispidus*, and perhaps this does contribute somewhat to the short times recorded in this particular test. Generally, however, the time taken to produce a given reaction was shorter than that recorded elsewhere, especially in the tests for emulsin, hemicellulase, and diastase.

Only the principal enzymes were investigated.

*Emulsin.* This enzyme has been known since 1837, and was found in 1894 in 34 species of fungi by Bourquelot, so that it appears to be generally distributed. Its importance in decay is that in the decomposition of wood, whether coniferous or otherwise, it is probable that various glucosides are set free.

Zeller found that in the decay of pine wood by *Lenzites*, vanillin was formed in fairly large quantities if the blocks were kept saturated with water. It is therefore probable that these glucosides, such as populin, arbutin, amygdalin, and coniferin, are broken down by the fungus and are partly converted to glucose, which is easily assimilated.

A 1 per cent. solution of amygdalin was used as a substrate, and 10 c.c. portions were placed in test-tubes. To three of these were added 1 c.c.

of enzyme extract No. 1 and to three 1 c.c. of No. 2. One of each of these was boiled. To three other tubes were added 1 c.c. of distilled water. To all were added toluene as an antiseptic. The tubes were incubated at 25° C. After 18 hours all the regulars gave a strong odour of benzaldehyde. The contents of the tubes were filtered off and tested for reducing sugars by means of Fehling's solution. All the regulars gave a positive result, while the controls gave a negative result. This shows that the mycelium of *P. hispidus* contains the enzyme emulsin.

*Diastase.* This enzyme has previously been reached in *Fomes annosus* by Hartig, and in other higher fungi by Bourquelot, especially in *Polyporus sulphureus*. It occurs in *Merulius lacrymans*, *Polyporus squamosus*, and *Armillaria mellea*, and Zeller has found it in both the mycelium and sporophores of *Lenzites saepiaria*.

A solution of soluble starch was used as a substrate in testing for this enzyme. It was prepared as follows:

2 gm. of starch in 100 c.c. of distilled water was brought to the boil, with constant shaking, and poured into 300 c.c. of hot distilled water. The resulting solution was then boiled for 2 hours under a reflux condenser, cooled, and brought up to 500 c.c.; to this was added toluene as an antiseptic.

To portions of the above solution in test-tubes were added: (1) enzyme suspension No. 1; (2) enzyme suspension No. 2; (3) enzyme suspension No 1 (boiled); (4) distilled water. They were incubated overnight at 25° C. and tested with Fehling's solution in the morning. Nos. 1 and 2 gave a considerable amount of reduction, while the two controls gave clear tests. Hence the mycelium of *Polyporus hispidus* contains the enzyme diastase.

*Invertase.* A 1 per cent. solution of cane sugar was used as a substrate, and to portions in test-tubes were added the enzyme extract, the extract boiled, and distilled water. The tubes were incubated at 25° C. as usual. No action was observed until after 18 hours, and even then it was very slight.

The mycelium undoubtedly does contain the enzyme *invertase*, though in very small quantity. This is more or less to be expected, since it is difficult to see where a wood-destroying fungus could obtain sucrose. The only possible place appears to be in the sap wood, where sucroses are probably present. Both Baxter and the writer have shown that *P. hispidus* can attack sap wood.

*Cytohydrolysing enzymes.* Under this general term will be included those enzymes which break up the higher carbohydrates, such as those which compose the cell-walls of plants. They include ligninase, cellulase, hemicellulase, pectinase. It has been known for a long time that fungi can effect a change in the properties of the lignified cell-wall, the first to call attention to this being Hartig, who showed that many species of fungi can break down the cell-walls of wood, and who stated that this was brought about by the action of a fluid secreted by the fungus.

Basidiomycetes are the fungi most concerned with the breaking down of the cell-walls, though under suitable conditions other groups can also attack wood. Miyoshi<sup>(12)</sup> and Marshall Ward<sup>(18)</sup> both showed that *Penicillium* spp. could attack wood, and the writer has frequently isolated species of *Penicillium* from woods which have been quite considerably decayed, and which, apparently, contained no other fungus. This has been especially noted in connection with a *Penicillium* which has been isolated, several times, from wood attacked by *Xestobium rufovillosum*.

While it is relatively easy to form the conclusion that a fungus which breaks down lignified walls must possess a lignin-splitting enzyme, it is by no means easy to devise a test by which the ligninase can be demonstrated.

Czapek<sup>(9)</sup> found that when decayed wood was extracted with hot alcohol or benzene, a resinous substance could be isolated from the alcohol, which he called "hadromal," probably an aldehyde, possibly coniferyl aldehyde. This he obtained from wood decayed by *Merulius lacrymans*, *Polyporus adustus*, *Pleurotus pulmonarius*, *P. ornatus*, and *Armillaria mellea*. From sound wood he obtained relatively little hadromal. He also noted that normal lignified walls show no sign of the cellulose reaction, but that when they are slightly decayed the zinc-chloride test for cellulose gives a positive reaction, though the walls show no signs of breaking down. He also found that the hadromal solution would give a pink coloration with phloroglucin and hydrochloric acid.

His conclusions were that the cell-wall is composed of a "cellulose-hadromal-ether." By the action of the enzyme produced by the fungus this ether is broken down, setting free the hadromal, which is soluble in hot absolute alcohol, and also liberating the cellulose, which can then give its characteristic colour reaction.

He prepared an enzyme suspension from *Merulius lacrymans* and *Pleurotus pulmonarius*, and incubated shavings in it for a fortnight. The extract from these shavings gave a strong hadromal test, and the cell-

walls of the shavings gave a purple reaction with zinc-chlor-iodide. The extract lost its activity when boiled.

Apart from the theoretical question of the validity of Czapek's conception of the lignin complex, it appears that a convenient test for an enzyme attacking lignified tissue is the reaction of the alcoholic extract of the decayed wood with phloroglucin and hydrochloric acid. Zeller used Czapek's test for "hadromase" as follows: he placed 1 gm. of shavings of *Pinus echinata*, previously soaked in distilled water, in test-tubes, and to these he added the enzyme dispersions. After 15 days the dispersions were poured off, and the shavings boiled in absolute alcohol, and the extract tested with phloroglucin and hydrochloric acid. The shavings acted on by the enzyme gave pink extracts, while the controls gave clear, colourless tests. Hence Zeller concluded that "these reactions show conclusively that hadromal is split off in the presence of an enzyme suspension from the mycelium of *Lenzites saepiaria*."

He attacks Czapek's nomenclature of the enzyme (hadromase) on the ground that it attacks lignin, not hadromal, and proposes that the term ligninase should be adopted. As this agrees with the terminology of Duclaux, which is in general use, it will be used here.

Baxter attempted to apply Czapek's test to distinguish between sound and apparently sound wood of *Fraxinus nigra* attacked by *P. hispidus*. He found that the reaction was given more or less equally by both the sound and the decayed wood. He prepared enzyme extracts in the usual way, and tested the sound wood against that acted on by the enzymes, but obtained no significant difference. His conclusion was that the results of Czapek's test cannot be relied upon, at any rate in the case of the hard woods.

The great discrepancy between the accounts of the reaction by these two workers makes it necessary that the matter should be cleared up. The fact that they worked with different woods has to be taken into account. In order to do this samples of sound wood of the following species were collected, and shavings made from them: *Pinus sylvestris*, *P. excelsa*, *Abies pectinata*, *Fraxinus excelsior*, *Castanea sativa*, *Fagus sylvatica*, *Salix* sp.

Small samples of these shavings were boiled for 10 minutes in absolute alcohol, and the extract carefully filtered, in order to remove small particles of wood. The alcoholic extracts were treated with phloroglucin and hydrochloric acid, and in every case a fairly deep red colour was produced. Wood of *Fraxinus excelsior* which had been decayed by *P. hispidus* for 5 months was treated in a similar manner. The difference

in the tests for sound and decayed wood was so small as to render the test valueless.

Hence it is clear that sound wood of the preceding species contains enough alcohol-soluble material, giving the phloroglucin test, to invalidate any estimate of the power of a fungus to attack the lignified tissues of the wood.

Attempts were made to treat the wood in such a way as to extract this substance. It was found that this can be done by boiling the wood in several changes of distilled water. When dried, and subsequently extracted with boiling alcohol in the usual way, the alcoholic extract is free from such substances giving the phloroglucinol reaction. Owing, however, to the probability that the hydrolysis of some of the wood compounds might take place during the boiling, this method was abandoned and the following adopted.

Shavings of the various woods were placed in large boiling tubes, and extracted with absolute alcohol under a reflux condenser. The alcohol was renewed from time to time, till the last traces of the substance giving the phloroglucin test was removed. That is, until the last extract did not give any perceptible coloration with phloroglucin and hydrochloric acid. Some of the woods gave a colourless test after three periods of extraction of 1 hour each, while the others needed as many as seven extractions of a similar time to remove all the "hadromal." The shavings were then dried, and kept in an air-dry condition as samples of wood with the hadromal of Czapek removed. Some of these shavings were soaked in distilled water for some time in order to remove the water-soluble materials, dried, and placed in boiling tubes. These were plugged, and sterilised by being subjected to a dry heat of 105° C. for 2 days. This temperature was chosen because no change in the chemical nature of wood takes place until the temperature exceeds this figure. Sterile water was then added to the tubes, and they were put on one side for several days until the wood had absorbed all the water it could.

The moist shavings were then transferred to the surface of agar slants which were covered with actively growing mycelium of *P. hispidus*, and incubated for 1 month.

The fungus attacked the shavings readily, but mycelial growth did not appear to be quite so vigorous as on untreated shavings exposed to the same conditions. At the end of 1 month the shavings were removed, dried, and each batch extracted with boiling alcohol for 10 minutes. The extracts were tested in the usual way with phloroglucin and hydrochloric acid, the whole seven, both from conifers and hard woods, giving a pink

coloration. Treated shavings, kept moist for a similar period, but not exposed to the action of the fungus, gave uniformly colourless results. Hence Czapek's hadromal reaction is not a suitable means of distinguishing between sound and decayed woods, since sound wood contains enough hadromal to give a very distinct reaction.

This hadromal contained in sound wood renders the method valueless for the purpose of testing for ligninase, the pink coloration of the alcoholic extract being given both before and after exposure to the fungus. The wood being treated in some such way as that described above, in order to remove the hadromal already present, the reaction becomes a valid test for ligninase.

In this way it is shown that the mycelium of *Polyporus hispidus* contains a ligninase capable of attacking the lignified tissues of the wood.

*Hemicellulase.* As the hemicelluloses are often a constituent part of the cell-wall, amounting, on the average, to from 9 to 12 per cent. of the dry weight of wood, it is therefore possible that they may play a part in the nutrition of a wood-destroying fungus.

Schellenberg has differentiated between those enzymes which resolve cellulose, and those which attack the hemicelluloses, and has also shown that some of the hemicellulases are specific in their activities. The hemicelluloses are more easily hydrolysed than the true celluloses, and also differ in their decomposition products. While the celluloses are hydrolysable to glucose, the hemicelluloses yield other sugars. The products of hydrolysis are sometimes pure sugars, and sometimes a mixture, the chief sugars to be obtained being mannose, dextrose, galactose, xylose, and arabinose. The nomenclature of the hemicelluloses is based on the sugars given on reduction.

The hemicellulose used in these experiments was paragalactan, which forms the greater part of the endosperm of the date (*Phoenix dactylifera*).

Date stones were thoroughly washed, and their outer coats removed by grinding on a small emery wheel. They were then cracked open, the embryos cut out, and the remaining endosperm autoclaved in order to deactivate any enzymes present. They were kept for future use in distilled water to which a trace of toluene had been added. Very thin slices of the paragalactan prepared as above were cut on a microtome, and small pieces of these were suspended in hanging drops in Van Tieghem cells.

Enzyme suspension No. 1 was used in these cells, and two sets of controls were used. The first was distilled water, while the second was the enzyme suspension after it had been deactivated by autoclaving.



A small quantity of toluene was added to all the cells as an antiseptic. Erosion started after 1 month, but was not very noticeable. After 2 months distinct erosion was seen in all the cells with the enzyme. In no case was the destruction of the endosperm so marked as in the similar pieces described by Zeller. There was no erosion at all in any of the controls.

All the drops remaining after the two months were tested for the presence of bacteria, but were found to be free from contamination.

In order to confirm these results some of the endosperms were ground to a fine meal on a rasp, and about 0.3 gm. placed in each of twelve test-tubes. These were divided into three batches.

1. Hemicellulose + Enzyme No. 1 + Toluene
2. „ + Enzyme No. 2 + „
3. „ + Enzyme No. 1 + „  
(autoclaved)
4. „ + Distilled water + „

They were all incubated at 25° C. for 14 days. The liquid was then filtered off, and tested for reducing sugars with Fehling's solution. Batches No. 1 and No. 2 gave strongly positive results, while the controls gave clear solutions.

Hence it is clear that the fungus *Polyporus hispidus* contains hemicellulase, capable of hydrolysing the hemicellulose paragalactan to galactose and arabinose.

*Oxidase.* The presence of oxidase was tested for, using a solution of guaiacum as an indicator.

Enzyme Suspension No. 1. To 3 c.c. of the extract in a test-tube was added three drops of hydrogen peroxide and five drops of a 1 per cent. solution of guaiacum. A blue coloration was produced in 4 minutes.

Enzyme Extract No. 2. To 1 c.c. of extract was added 3 c.c. of distilled water, four drops of hydrogen peroxide and five drops of guaiacum. A blue colour was produced in  $3\frac{1}{2}$  minutes.

Distilled water and boiled enzyme extracts gave no coloration at all. Hence it is concluded that there is a considerable quantity of oxidase in the mycelium of *Polyporus hispidus*.

*Catalase.* Catalase was tested for by adding the enzyme extract to hydrogen peroxide.

Extract No. 1. When added to hydrogen peroxide some effervescence took place, showing that oxygen was being liberated.

Extract No. 2. When added to hydrogen peroxide a great deal of frothing occurred, so much that the test-tube was filled with the foam produced by about 1 c.c. of extract and 1 c.c. of peroxide.

Distilled water and boiled extract produced no frothing, showing that the mycelium of *P. hispidus* contains an oxidase.

#### SUMMARY.

The characteristics of the *Polyporus hispidus*, when grown on artificial media, both solid and liquid, are described and compared with those given by Baxter.

Growth on wood of ash under laboratory conditions produces a rot which is indistinguishable from that occurring naturally. The distribution of the hyphae in the wood is described.

The mode of penetration of the cell-wall by the hyphae is figured. It is apparently by pits in the early stages of decay, but by bore-holes, formed entirely by enzyme activity, in the more advanced rot.

The rate of growth of the fungus under controlled conditions has been measured, and shown to be 0.5 cm. per month.

Successful inoculation experiments have been carried out with young trees, confirming the results of Baxter, who states that the fungus can attack young, living sap wood.

An investigation of the enzymes produced by the mycelium has been carried out, and a method evolved for demonstrating the presence of a ligninase. This is a modification of Czapek's "hadromal" reaction.

The following enzymes are shown to be present in the mycelium: emulsin, diastase, invertase, ligninase, hemicellulase, oxidase, and catalase. The list is not intended to be exhaustive.

The writer desires to express his thanks to Mr W. R. Day, of the Imperial Forestry Institute, Oxford, and to Dr W. Brown, of the Royal College of Science, for helpful criticism and advice. Also to Mr R. S. Pearson, C.I.E., F.L.S., the Director of Forest Products Research, for permission to publish this paper.

#### REFERENCES.

- (1) BAXTER, D. V. The biology and pathology of some hardwood heart-rotting fungi. *Amer. Journ. Bot.* xii, Nos. 8 and 9.  
— The heart rot of Black Ash caused by *Polyporus hispidus* Fr. *Papers Mich. Acad. Sc. Arts and Letters*, III.
- (2) BERTRAND, G. Sur une nouvelle oxydase, ou ferment soluble oxydant, d'origine végétale. *Compt. rend. Acad. Paris*, cxxii, 1215-1217.
- (3) BOURQUELOT. Présence d'un ferment analogue à l'émulsine dans les champignons, et en particulier dans ceux qui sont parasites des arbres, ou vivent sur les bois. *Bull. Soc. Myc. Fr.* ix.

- (4) BOURQUELOT et HERISSEY. Les ferments solubles du *Polyporus sulphureus*. *Bull. Soc. Myc. Fr.* XII, 18-26.
- (5) BOYCE, J. S. Decays and discolorations in airplane woods. *U.S. D.A. Bull.* No. 1128.
- (6) BROWN, W. Studies in the Physiology of Parasitism. I. The action of *Botrytis cinerea*. *Ann. Bot.* XXIX, 313-348.
- (7) BULLER, A. H. R. The enzymes of *P. squamosus* Huds. *Ann. Bot.* XX, 49-59.
- (8) COLLEY, R. H. The effect of incipient decay on the mechanical properties of airplane wood. *Phytopath.* XI, 45.
- (9) CZAPEK. Zur Biologie der holzbewohnenden Pilze. *Ber. d. deut. Bot. Ges.* CLXXI, 166-170.  
 — Ueber die sogenannten Ligninreactionen des Holzes. *Zeit. für Physiol. Chem.* XXVII, 141-166.  
 — *Biochemie der Pflanzen*. Jena, 1913.
- (10) HUBERT, E. E. The effect of kiln-drying, steaming, and air-seasoning on certain fungi in wood. *U.S. D.A. Bull.* No. 1262.  
 — Diagnosis of decay in wood. *Journ. Agric. Res.* XXIX, 523.
- (11) LINDROTH, J. I. Beiträge zur Kenntnis der Zersetzungserscheinungen des Birkenholzes. *Naturw. Zeit. Land- u. Forstw. Jahrb.* II.
- (12) MIYOSHI, M. Die Durchbohrung von Membranen durch Pilzelfäden. *Jahrb. für Wiss. Bot.* XXVIII, 269-289.
- (13) MURRELL, W. A. *Northern Polypores*.
- (14) PRILLEAUX, E. *Polyporus hispidus*. *Bull. Soc. Myc. Fr.* 255-259. 1893.
- (15) REA, CARLETON. *British Basidiomycetae*.
- (16) SCHEELENBERG, H. C. Untersuchungen über das Verhalten einiger Pilze gegen Hemicellulose. *Flora*, xcvi, 257-308.
- (17) SCHMITZ. Enzyme action in *Polyporus volvatus* (Peck) and *Fomes igniarius* (L). *Journ. Gen. Physiol.* II, 613-616.
- (18) WARD, M. *Penicillium* as a wood-destroying fungus. *Ann. Bot.* XII, 565-566.
- (19) WESTERDIJK, J. *Report of the International Conference of Phytopathologists and Economic Entomologists, Holland.* 1923.
- (20) ZELLER, S. M. Physiology of *Lenzites saepiaria*. *Ann. Mo. Bot. Gard.* III, 439-512.

## EXPLANATION OF PLATES VI—VIII.

### PLATE I.

- Fig. 1. Camera lucida drawing of ash wood after 4 months' attack by *Polyporus hispidus*. Note that the penetration of the cell-wall is almost entirely through the pits.

### PLATE II.

- Fig. 2. Camera lucida drawing of ash wood after 6 months' attack by *Polyporus hispidus*. The shaded cells to the left of the drawing are full of large empty brown hyphae, and form the edge of the zone line. Note the penetration by bore-holes, as in Text-fig. 2, and the large dead hyphae scattered about in the vessels.
- Fig. 3. Drawing of surface of wood attacked by *Polyporus hispidus*, under the ring illuminator. The mycelium in the vessels is drawn with the camera lucida, while the wood is semi-diagrammatic. Note the considerable development of mycelium in the vessels; this would be washed out if sections were cut.

### PLATE III.

- Fig. 4. Section of trunk of 85-year-old ash tree attacked by *Polyporus hispidus*. The rot produced can be seen in the centre. In this case the probable path of entry of the fungus was by the branch stub shown.
- Fig. 5. "Bore-hole" of *Polyporus hispidus* in ash. × 1200.

(Received June 2nd, 1928.)

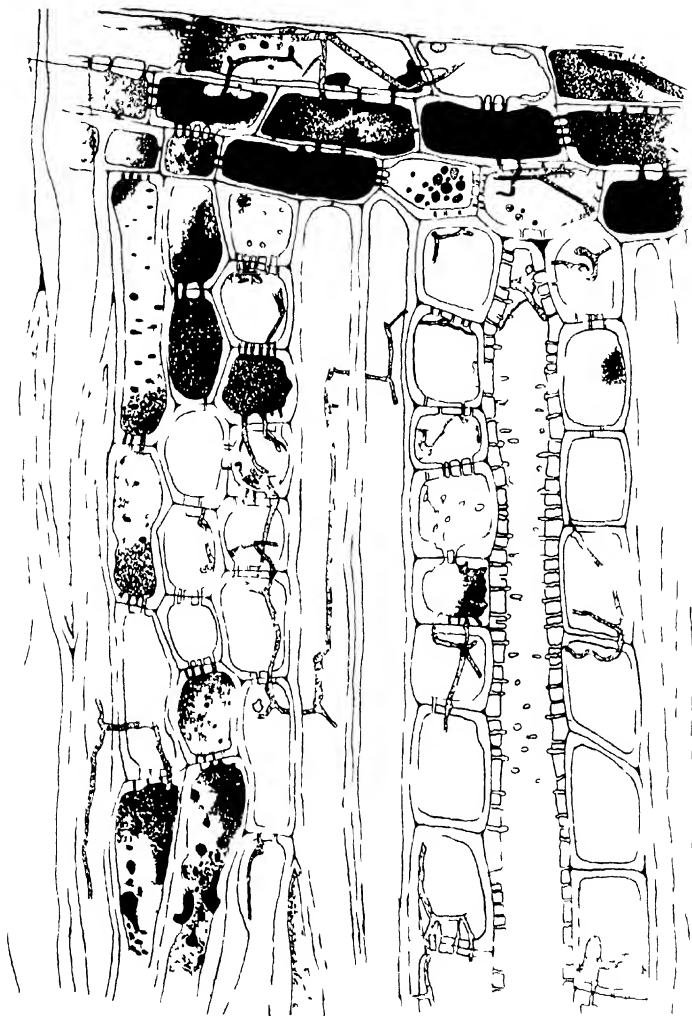


Fig. 1

NUTMAN.—STUDIES OF WOOD-DESTROYING FUNGI (pp. 40-64)





Fig. 2

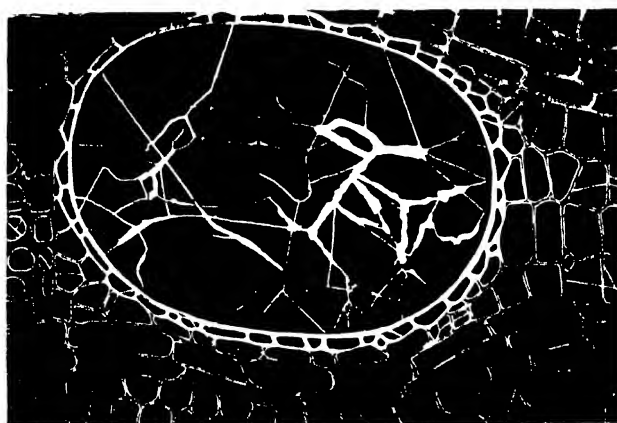


Fig. 3.

NUTMAN.—STUDIES OF WOOD-DESTROYING FUNGI (pp. 40-64).





Fig. 4.

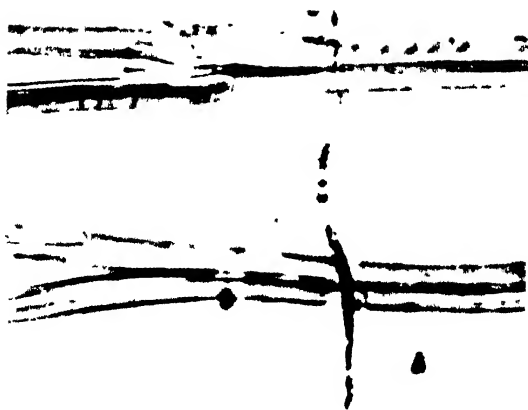


Fig. 5.

NUTMAN.—STUDIES OF WOOD DESTROYING FUNGI (pp. 40-64).





# THE BIOLOGY OF OAT SMUTS

## II. VARIETAL RESISTANCE

By KATHLEEN SAMPSON, M.Sc. (LOND.).

(*University College of Wales, Aberystwyth.*)

(With Plate IX and 1 Text-figure.)

### CONTENTS.

	PAGE
I. INTRODUCTION . . . . .	65
II. METHODS OF TESTING OAT VARIETIES FOR RESISTANCE TO SMUT . . . . .	68
1. Shelled grain <i>versus</i> grain in husk . . . . .	68
2. Influence of different dates of sowing under field conditions . . . . .	68
3. Infection produced under controlled conditions of temperature and moisture . . . . .	70
4. Infection caused by contaminating the open flowers . . . . .	72
III. FURTHER BIOLOGICAL SPECIES OF <i>U. AVENAE</i> AND <i>U. LEVIS</i> . . . . .	75
IV. THE RELATIVE RESISTANCE OF LINE SELECTIONS OF <i>AVENA STRIGOSA</i> TO <i>U. LEVIS</i> , BIOLOGICAL SPECIES C 1 . . . . .	79
V. OBSERVATIONS ON THE NATURAL SMUT INFECTION OF CERTAIN BRITISH OAT VARIETIES . . . . .	80
VI. SUMMARY . . . . .	83
VII. ACKNOWLEDGMENTS . . . . .	84
VIII. REFERENCES . . . . .	84
EXPLANATION OF PLATE. . . . .	85

### I. INTRODUCTION.

PROOF of the existence of biological specialisation in loose and covered smuts of oats was given by Reed (9) in 1924, and by Sampson (13) in the following year. Each writer worked with the same races, and the results obtained on a number of oat varieties in Missouri and at Aberystwyth showed substantial agreement, leaving no doubt as to the validity of the distinctions drawn between the four races under study.

In 1927 Reed (10) published data which gave further evidence of biological specialisation in oat smuts. He worked with collections of chlamydospores of *Ustilago avenae*, which differed from forms previously studied in their capacity for attacking varieties of the species *Avena sterilis*. Reed found that the collections belonged to two distinct biological species, the

one capable of attacking selections of Fulghum but giving no infection on Red Rustproof, while the second gave exactly contrary results, attacking Red Rustproof but not Fulghum.

There are therefore at present four well-defined biological species of *U. avenae* and two of *U. levis*. They can be distinguished by suitable varieties or selections of the hosts indicated in the list below.

Country of origin	Susceptible	Resistant
<i>U. avenae</i> —		
1. Wales	<i>A. sativa</i> , Potato, Radnorshire sprig, and others	<i>A. strigosa</i> , <i>A. nuda</i> , <i>A. sterilis</i>
2. U.S.A.	<i>A. sativa</i> , <i>A. strigosa</i> , <i>A. nuda</i>	<i>A. sterilis</i>
3. U.S.A.	<i>A. sterilis</i> , Fulghum	<i>A. sterilis</i> , Red Rustproof
4. U.S.A.	<i>A. sterilis</i> , Red Rustproof	<i>A. sterilis</i> , Fulghum
<i>U. levis</i> —		
1. Wales	<i>A. strigosa</i> , <i>A. brevis</i>	<i>A. sativa</i> , <i>A. nuda</i> , <i>A. sterilis</i>
2. U.S.A.	<i>A. sativa</i> , <i>A. strigosa</i> , <i>A. nuda</i>	<i>A. brevis</i> , <i>A. sterilis</i>

During the past three years it has been possible to study in Wales the infection capacities of certain other spore collections of both species of oat smut. The results indicate that two more biological species must be added to the above list. The first is a race of *U. avenae* (L 11)<sup>1</sup> which has been found capable of attacking only varieties of *A. brevis* and *A. strigosa*, from which species it was originally isolated. The second is a race of *U. levis* (C 3), which was obtained from *A. sativa*, Grey Winter in England. This race shows a decided similarity to the Missouri strain of *U. levis* (C 2) first studied by Reed (9), but it differs from the latter in that it gives low or negative results on *A. strigosa orcadensis* (Cc 521) and on *A. nuda* (Cc 2495), whereas these varieties are highly susceptible to the Missouri strain. The experimental data are discussed below.

With the object of obtaining data relating to the percentage attack on commercial varieties, over two hundred samples of oats from different districts in the British Isles were grown at the Station in 1927. The results are interesting in connection with the general problems of varietal resistance and distribution of biological species. Not a single sample of *A. sativa* showed any infection from *U. levis*, a result which confirms the previously expressed belief as to the rare occurrence in Britain of strains of this species capable of attacking *sativa* varieties (13). The results also agree with those of Stapledon (16) which indicated that in this country

<sup>1</sup> References in brackets indicate the index numbers given by the author to the collections of spores under study. The letters L and C denote the species *U. avenae* and *U. levis* respectively. Numerals refer to particular strains, and small letters have been used to distinguish collections of spores harvested on different dates or by different methods.

infection is usually heaviest on the older varieties such as are included in the Winter, Potato and Sprig groups<sup>1</sup>. It is interesting to find that the same varieties were among the most susceptible of those tested experimentally with the Welsh strain *U. avenae* (L 1) isolated originally from Potato oats. It is probable that this represents a biological species widely distributed in the British Isles.

A microscopic examination was made of the pales of grain from samples which produced a relatively high percentage of infection in the field. From this it was evident that "flowering infection" as defined by Zade(19) was of general occurrence. It follows that the extent to which oat varieties open their pales and the atmospheric conditions during and shortly after the flowering period, must be counted among the important factors which determine the intensity of the smut attack in the subsequent crop. It is not improbable that a variety which gives high infection figures when tested under special experimental conditions will not necessarily fall among the most susceptible varieties when natural infection is in question. It is possible that some of the newer varieties are largely "smut escaping" though not strongly "smut resistant."

Table I.

*Showing the incidence of smut on shelled and hulled grain of certain oat varieties. Sown March 13th, 1925. Farm cage. Reference C 138, I.*

Variety	Station number	Grain in husk				Grain shelled			
		Number of plants	Smutted plants %	Number of panicles	Smutted panicles %	Number of plants	Smutted plants %	Number of panicles	Smutted panicles %
I. Loose smut ex <i>A. sativa</i> (L 1)—									
<i>A. strigosa glabrescens</i>	363	210	0	316	0	202	0	308	0
<i>A. sativa</i> , Black Bell	2475	119	0	141	0	131	0	165	0
„ Orion	2477	135	0	150	0	144	0	200	0
„ Record	1642	165	0	232	0	99	7.1	150	6.0
„ Ceirch du bach	1080	182	0.6	252	0.4	175	12.6	287	10.8
„ Potato	1029	161	1.9	190	3.2	51	45.1	81	37.0
II. Covered smut ex <i>A. strigosa</i> (C 1)—									
<i>A. strigosa pilosa</i>	362	207	5.8	260	5.4	200	51.0	360	58.3
„ <i>glabrescens</i>	363	179	5.0	229	5.2	154	47.4	185	36.8
<i>A. brevis</i>	1614	200	0.5	342	0.3	68	2.9	133	1.5
<i>A. sativa</i> , Record	1642	169	0	214	0	110	0	147	0
„ Ceirch du bach	1080	196	0	259	0	181	0	275	0

<sup>1</sup> Here and elsewhere in the paper the grouping of varieties follows the classification given by Marquand (9).

## II. METHODS OF TESTING OAT VARIETIES FOR RESISTANCE TO SMUT.

Resistance tests carried out during the seasons 1922 to 1924 with heavily contaminated grain sown under field conditions gave on the whole remarkably low infection, and it became necessary to modify the technique adopted in these early experiments (13). The different methods which have been tested are described below. The spore material was obtained from previous experiments and it included four of the biological species which have been already defined with reference to their infection capacities on certain oat varieties.

### 1. *Shelled grain versus grain in husk.*

In oats and barley (5, 17, 3) the chances of infection are considerably increased by removing the pales and applying chlamydospores to the surface of the caryopsis<sup>1</sup>. Some results obtained by sowing in the open shelled and hulled grain of certain oat varieties contaminated with two biological species of *U. avenae* and *U. levis* are summarised in Table I. The increase due to removal of the husk was in the case of *U. avenae* on Potato as much as 33.8 per cent., and in the case of *U. levis* on *A. strigosa pilosa* 42.9 per cent. It is important to notice that the use of shelled grain emphasises the differences between the biological species; susceptible varieties show considerably increased infection, but resistant varieties continue to give negative results. Johnston (5) arrived at a somewhat similar conclusion.

Further evidence of the distinct advantage of shelled grain in experimental work of this kind is given in Tables III, IV and VII. In later experiments (Tables V and VI) only shelled grain was used.

### 2. *Influence of different dates of sowing under field conditions.*

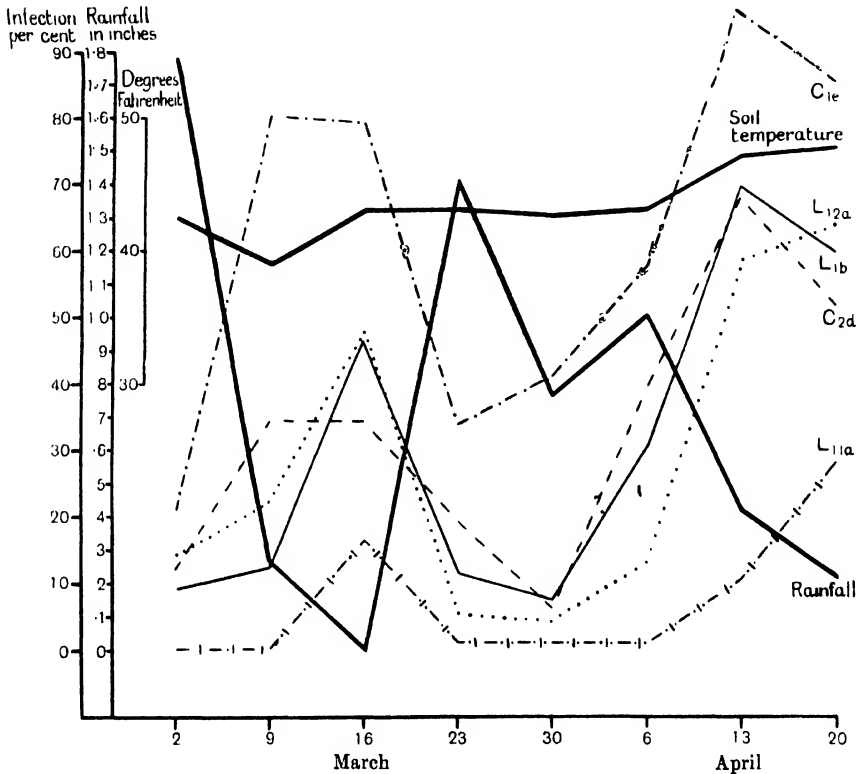
Several investigators (2, 5, 11, 18) have shown that the intensity of the attack of smut fungi in oats is influenced considerably by the temperature and by the moisture content of the soil during the period of germination. All agree that relatively dry soil and a moderately high temperature favour infection.

In 1927, starting on February 28th, eight sowings were made at weekly intervals of shelled grain contaminated with five biological species of smut. Suitable susceptible varieties of oats were used as hosts for the different races of smut. Each sample was sown in duplicate at the rate

<sup>1</sup> The method of obtaining chlamydospores free from fragments of the host plant is described in an earlier paper (14).

of 150 grains per 5-foot row. The ground was uniform and the experiment covered only a small area.

The percentage infection of the different lots is represented graphically in Fig. 1, together with the average temperature of the soil for the



- Rainfall in inches. Average of week, 2 days before to 5 days after sowing.
- Soil temperature at 9 a.m. Depth 4 inches. Average of week after sowing.
- . | . — . | . — *U. avenae* L11a on *A. strigosa glabrescens*. 1706 Ce 5.
- *U. avenae* L1b on *A. sativa*. Potato 3191.
- ..... *U. avenae* L12b on *A. sativa*. Potato 3191.
- . . . . . *U. levis* C1e on *A. strigosa orcadensis*. 3190.
- — — — — *U. levis* C2d on *A. sativa*. Potato 3191.

Fig. 1. Graph showing the percentage infection from weekly sowings of oats contaminated with five spore-collections of loose and covered smut. Shelled grain. Phenological Garden, 1927.

week following each date of sowing, taken at a depth of 4 inches. The total rain which fell during the interval, 2 days before to 5 days after sowing, is also shown in the graph. This may be taken as a general

indication of the changes which occurred in the moisture content of the soil during the experimental period.

The intensity of attack produced by the different biological species showed a wide range of variation. *U. levis* (C 1) varied between the limits 34 to 95 per cent. on the different dates of sowing. This high infection is probably due in part to the heavy contamination of the grain and to the good viability of the spores. *U. avenae* (L 11) gave on the other hand low infection, the figures ranging from 0 to 28 per cent. This was probably caused by the poor viability of the spores which gave a germination of only 2 per cent., and by the fact that a smaller bulk of material was available for contamination (Fig. 1).

The two species, *U. avenae* and *U. levis*, showed on the whole the same general response to changes in the conditions which operated on the grain which was sown on different dates. The graphs for the five individual samples show two peaks which appear to be correlated with two periods of low rainfall. The temperature of the soil did not vary widely during the first seven weeks of the experimental period, and rainfall rather than temperature appears to have been in this experiment the critical factor in determining the relative intensity of the attack. That a relatively low moisture content of the soil favours infection of oats by both species of smut has been shown by others who have made experiments under artificial (2, 11, 18) or natural conditions (5). The present experiment supports this view.

### *3. Infection produced under controlled conditions of temperature and moisture.*

The data discussed in the previous section explain the uncertainty of resistance trials conducted under conditions which are subject to vagaries of the weather. It is clearly important that both temperature and humidity should be under control when the grain germinates.

A preliminary trial in 1924 showed that it is only necessary to control these factors for a short period, and the following method was adopted in 1925 and in subsequent seasons. Certain details of technique were suggested by the work of Reed (9).

*Germination on sand (Method C).* Uniform, dry and sterilised sand was mixed with sufficient water to give a moisture content equal to 20 per cent. of its saturation capacity<sup>1</sup>. Soaked earthenware saucers, glazed on the inside, were partly filled with sand. Shelled grain coated with spores was sown on the smoothed out surface and covered uniformly

<sup>1</sup> The sand used passed through a 1.0 mm. sieve, and had a pH value of 8.2.

with sand to a depth of 1 in. The dishes were placed in an incubator at 22° C. together with open dishes of water. The small quantity of water lost by the sand was replaced daily by spraying the surface until the dish regained its original weight. In most varieties the majority of the seedlings were visible above the surface by the fourth or fifth day. Each was marked by a small rubber ring and the dishes were placed on the greenhouse bench. Finally, marked plants were transferred to large pots or to boxes of soil in which they were allowed to mature.

In Table II the results are given of trials with four biological species. In series *a* and *b* the seedlings were planted in duplicate Doulton pots;

Table II.

*Showing the infection of shelled grain germinated in sand under controlled conditions of temperature and moisture. Two biological species of U. avenae and U. levis. 1925. Reference C 86.*

Species and variety of host	Ref. to series	Number of plants	Smut- ted plants %	Number of panicles	Smut- ted panicles %	Number of plants	Smut- ted plants %	Number of panicles	Smut- ted panicles %
		<i>U. avenae</i> ex Wales (L 1)				<i>U. avenae</i> ex U.S.A. (L 2)			
<i>A. nuda</i> (2495)	<i>a</i>	8	12.5	22	4.5	6	100	24	100
	<i>b</i>	12	0	20	0	8	100	25	100
	<i>c</i>	11	0	28	0	3	100	8	100
	Average		4.2		1.5		100		100
<i>A. sativa</i> , Sandy (2448)	<i>a</i>	9	0	32	0	9	11.1	39	7.7
	<i>b</i>	9	0	35	0	9	33.4	36	38.9
	<i>c</i>	8	0	26	0	8	25.0	24	16.7
	Average		0		0		23.2		21.1
<i>A. strigosa orcadensis</i> (2662)	<i>a</i>	10	0	71	0	8	50.0	80	45.0*
	<i>b</i>	11	0	70	0	9	88.9	75	76.0*
	<i>c</i>	8	0	36	0	5	80.0	28	78.6*
	Average		0		0		73.0		66.5
		<i>U. levis</i> ex Wales (C 1)				<i>U. levis</i> ex U.S.A. (C 2)			
<i>A. sativa</i> , Potato (1029)	<i>a</i>	9	0	32	0	7	28.6	27	25.9†
	<i>b</i>	10	0	32	0	8	50.0	23	56.5
	Average		0		0		39.3		41.2
<i>A. strigosa orcadensis</i> (2662)	<i>a</i>	9	100	56	100	10	80.0	63	68.2
	<i>b</i>	11	100	60	100	10	70.0	76	59.2
	<i>c</i>	4	75	13	69.2	8	62.5	34	55.8
	Average		91.7		89.8		70.8		61.1

\* On this host the biological species L 2 developed slowly, infecting the glumes and pales only to a slight extent and retarding growth comparatively little (Pl IX, fig. 1 (d)).

† One plant showed sori of chlamydospores on the flag-leaves of four tillers.



in series *c* large wooden boxes were used. The data for each series are given separately in order to show that the method gives concordant results with relatively few plants per unit. In later tests by this method the results given represent the average from two pots each of which contained 9 to 11 plants (Tables V and VI).

Plate IX, fig. 1, shows the appearance of four pots of *A. strigosa* infected by four distinct biological species of smut. The races differ not only in the number of plants with smutted panicles (Table II) but also in their influence on the growth of the host plant. *U. levis* (C 1), which gave 100 per cent. infection, also caused the greatest reduction in height (pot *a*).

This method was equally satisfactory in 1926 and 1927, duplicate pots giving close agreement.

*Germination on filter-paper.* In 1927 certain samples of grain, shelled and shaken with chlamydospores, were sown in rows on double sheets of damp filter-paper, which were rolled up and placed on the shelf of an incubator running at 22° C. After 3 days the germinated seedlings were planted in moist soil in boxes and left until maturity. The results obtained with fourteen samples (Tables V and VI) agreed closely with those from parallel tests in sand. Further trials of the method are in progress.

#### 4. *Infection caused by contaminating the open flowers.*

In 1925 an attempt was made to test the resistance of a number of varieties to selected strains of smut, by placing chlamydospores between the pales when the plants were in flower, a method which was recommended by Zade(19) as giving higher infection than that which usually followed the sowing of contaminated grain in spring. Preliminary observations showed that oat varieties varied widely in the extent to which their pales opened, and in order to test a large number of varieties the spores were placed between pales, temporarily separated by means of forceps. The danger of accidental contamination by air-borne spores was minimised by enclosing the panicles shortly after emergence in pollen-proof bags, and opening these only for the purpose of applying the spores. Grain from panicles enclosed but not inoculated gave, with one single exception, healthy plants in the following year.

From series A and B (Table III) spikelets were cut from time to time, fixed in 70 per cent. alcohol and examined microscopically. In some spikelets germinated spores were found on the second day after inoculation. In others, cut 2 to 9 days after inoculation, no sign of germination

was evident. The following figures indicate the number of spikelets in which germination had occurred 2 to 14 days after inoculation. Twenty spikelets were examined in L 1, C 1 and C 2, nineteen in the case of L 2. The host was *A. sativa*, Potato. *U. avenae* L 1, 20; *U. avenae* L 2, 17; *U. levis* C 1, 11; *U. levis* C 2, 20.

Germination counts were not possible, but it was evident that a relatively small number of the spores introduced between the pales had germinated.

Pales from the ripe grain were also examined microscopically at a later date. The grains were soaked in water for 12 hours and then fixed in alcohol. The pales were treated by the lacto-phenol and cotton blue method and mounted so that the inner surface of each pale could be examined.

Twenty-one grains from series C were examined by this method and thirteen showed mycelium or gemmae such as Zade (19,20) described (Plate IX, fig. 2 (c)). In some cases it was possible to trace a connection between the mycelium and germinated spores of *Ustilago*. It was evident that germination between the pales occurred in both species of smut and on both susceptible and resistant varieties.

Grain contaminated by the above method was sown in boxes in an unheated greenhouse on March 11th, 1926. Later the plants were spaced out in rows on the farm cage. Parallel tests were made with grain shelled and grain in the husk, which was shaken with spores which had been kept in store during the winter. The results for each series are given in Table III.

It should be stated in the first place that the positive results which the method of "flowering infection" gave in A I and B I are entirely in harmony with the infection capacities of the particular biological species under test, as determined by previous trials. The spore collection, L 10, isolated from Grey Winter, was then tested for the first time. It appears to resemble *U. avenae* L 1, isolated from Potato, but further work with this collection was not carried out.

Comparing the three methods of treatment, it is necessary to consider separately the series A, B and C. With two races of *U. levis* (series B), grain contaminated in spring gave higher infection than that obtained from flowers dusted with spores in the previous summer (Method I).

In series A, method I is sometimes superior, sometimes inferior to method II, the contamination of shelled grain. In series C, which involved a different collection of spores, method II gave completely negative results with the exception of a single infected plant of Earl Haig. Method I

Table III.

Showing the infection of certain oat varieties by three methods of contamination with *chlamydospores* of *U. avenae* and *U. levis*. 1925-26.  
Reference C 162 and C 148.

Species and variety of host	Ref.	I. Flowering period			II. Shelled grain			III. Grain in husk		
		No. of plants	No. of plants smutted	Smut- ted plants %	No. of plants	No. of plants smutted	Smut- ted plants %	No. of plants	No. of plants smutted	Smut- ted plants %
A. <i>U. avenae</i> —										
<i>A. sativa</i> , Potato	L 1	38	10	26	20	0	0	20	0	0
„ „	L 2	50	9	18	17	6	35	24	1	4
„ Sandy	L 1	26	0	0	17	0	0	15	0	0
„ „	L 2	27	15	56	21	11	52	21	9	43
<i>A. strigosa</i>	L 1	17	0	0	10	0	0	18	0	0
„	L 2	10	0	0	19	0	0	17	0	0
<i>A. nuda</i>	L 1	43	0	0	17	0	0	—	—	—
„	L 2	37	4	11	20	7	35	—	—	—
B. <i>U. levis</i> —										
<i>A. sativa</i> , Potato	C 1	55	0	0	22	0	0	23	0	0
„ „	C 2	51	6	12	21	7	33	21		19
<i>A. strigosa</i>	C 1	17	11	65	14	14	100	17	13	78
„	C 2	11	0	0	15	3	20	16	4	25
C. <i>U. avenae</i> —										
<i>A. sativa</i> , Ceirch du bach*	L 10	47	10	21	21	0	0	19	0	0
„ Potato*	„	35	4	11	18	0	0	18	0	0
„ Potato	„	14	5	35	—	—	—	—	—	—
„ Radnorshire	„									
„ sprig*	„	17	2	12	21	0	0	18	0	0
„ Grey Winter*	„	7	1	14	17	0	0	18	0	0
„ Earl Haig*	„	10	1	10	15	1	7	19	0	0
„ Earl Haig	„	10	1	10	—	—	—	—	—	—
„ Black Tartar	„	31	1	3	19	0	0	14	0	0
„ Marvellous	„	24	1	4	—	—	—	—	—	—
„ Record	„	19	0	0	16	0	0	19	0	0
„ Gelbhafer*	„	21	0	0	20	0	0	20	0	0
„ Markton*	„	2	0	0	19	0	0	23	0	0
„ Captain	„	15	0	0	17	0	0	12	0	0
„ Golden Rain	„	8	0	0	21	0	0	15	0	0
„ Waverley	„	27	0	0	15	0	0	15	0	0
„ Crown	„	18	0	0	20	0	0	19	0	0
„ Leader	„	24	0	0	21	0	0	19	0	0
„ Abundance	„	19	0	0	18	0	0	21	0	0
„ King	„	18	0	0	20	0	0	20	0	0
„ Victory	„	16	0	0	18	0	0	15	0	0
<i>A. strigosa orcadensis</i> *	„	61	0	0	20	0	0	19	0	0
<i>A. brevis</i> *	„	24	0	0	21	0	0	18	0	0

\* Contaminated by dusting *exserted* stigmas with *chlamydospores*. In other cases the spores were placed between the pales, which were artificially separated with forceps.

gave positive results in nine varieties, but in no case was the infection severe, the highest figure being 35 per cent. on Potato.

It is not improbable that the above differences are related to the viability of the chlamydospores. It has been shown in a previous paper<sup>(14)</sup> that the spores of *U. levis* remain viable in store for a considerable period, whereas chlamydospores of *U. avenae*, particularly when collected from immature plants, rapidly lose their power of germination. In the samples under discussion collections L 1 and L 2 gave low but appreciable germination in spring 1926, while L 10 lost all trace of viability as early as October 1925. Positive results were not to be expected from grain dusted with spores of this collection in March 1926. It is probable therefore that plants infected by L 10 (method I) owed their infection to mycelium or gemmae formed on the pales in the previous summer. Infection in series A and B (method I) may have been due either to such resting mycelium or to the germination of those chlamydospores which had retained their viability during the period of storage.

The method of contaminating flowers was distinctly laborious, and the results obtained are clearly less satisfactory than those given by shelled grain sown on sand as described in the previous section.

Arland<sup>(1)</sup>, Diehl<sup>(4)</sup> and Rosch<sup>(12)</sup> have tested the resistance of German varieties of oats by introducing chlamydospores already germinated, between the pales of ripe grain. High percentages of infection were seldom obtained, though the method was considered to be more reliable than the dusting of grain in husk.

### III. FURTHER BIOLOGICAL SPECIES OF *U. AVENAE* AND *U. LEVIS*.

The essential differences between the biological species L 1 and L 2 of *U. avenae*, and C 1 and C 2 of *U. levis*, have been clearly brought out in the experiments designed to test different methods of technique. The present section deals with four additional collections of spores, two of which possess infection capacities distinct from any biological species previously identified.

1. *U. avenae* (L 11) isolated from *A. strigosa glabrescens* in Wales.

(a) *Field experiment.* 1926. *Reference C* 166.

Thirty-two samples of oats were contaminated in March 1926 with chlamydospores of L 11 and L 12, two collections of *U. avenae* obtained from *A. strigosa glabrescens* and *A. sativa*, Potato respectively. With the exception of the varieties of Black Mesdag and Hull-less both shelled and hulled grain of each variety were tested. Each sample was sown in

Table IV.

Showing the behaviour of two biological species of *Ustilago avenae* on thirty-one varieties of oats.  
Sown March 27th, 1926. Upper Ridge Field. Reference C 166.

Station number	Variety	<i>Ustilago avenae</i> ex <i>Avena strigosa</i> Reference L 11				<i>Ustilago avenae</i> ex <i>Avena sativa</i> var. Potato Reference L 12			
		Shelled grain		Grain in husk		Shelled grain		Grain in husk	
		Total no. of panicles	No. smutted panicles %	Total no. of panicles	No. smutted panicles %	Total no. of panicles	No. smutted panicles %	Total no. of panicles	No. smutted panicles %
362	<i>A. strigosa pilosa</i>	221	189 93	244	131 53	152	0 0	196	0 0
621	" <i>orcadensis</i>	185	167 90	181	83 46	92	0 0	147	0 0
2877	" <i>glabrescens</i>	131	105 80	140	69 49	138	0 0	134	0 0
2384	<i>A. brevis</i>	117	78 66	183	114 62	180	0 0	162	0 0
2855	<i>A. sativa</i> , Potato	136	0 0	132	0 0	197	174 88	217	75 35
2798	" Captain	166	0 0	123	0 0	145	56 39	168	6 4
975	" Carth du bach	201	0 0	162	0 0	156	57 36	259	24 9
1103	" Radnorshire sprig	181	0 0	176	1 0.6	162	42 26	151	26 17
2799	" Bountiful	156	0 0	143	0 0	123	34 26	192	20 10
2854	" Tartar King	109	0 0	155	0 0	140	36 26	139	3 2
2860	" Grey Winter	152	0 0	172	0 0	134	19 14	193	3 2
2804	" Record	107	0 0	151	1 0.7	121	23 13	173	15 9
2810	" Yielder	143	0 0	124	0 0	121	11 9	151	9 6
2801	" Waverley	157	0 0	149	0 0	165	12 7	194	5 3
2806	" Black Tartar	156	0 0	121	0 0	132	6 5	127	2 2
2787	" Superb	124	0 0	135	0 0	132	3 2	184	0 0
2802	" Haig	135	0 0	170	0 0	165	3 2	168	1 0.6
2809	" Marvellous	132	0 0	142	0 0	138	1 0.7	173	0 0
2805	" Victory	145	0 0	125	0 0	174	1 0.6	192	0 0
2782	" Gelbhafer	159	0 0	187	0 0	163	0 0	217	0 0
2791	" Markton	136	0 0	164	0 0	134	0 0	183	0 0
2792	" King	175	0 0	159	0 0	168	0 0	192	0 0
2784	" Crown	151	0 0	155	0 0	130	0 0	192	0 0
2795	" Golden Rain	139	0 0	156	0 0	145	0 0	209	0 0
2796	" Ligowo	163	0 0	171	0 0	162	0 0	204	0 0
2800	" Leader	152	0 0	162	0 0	176	0 0	178	0 0
2807	" Abundance	148	0 0	178	1 0.6	210	0 0	183	0 0
2371	" Black Meadag	170	1 0.6	—	—	168	0 0	—	—
1610	" Black Meadag	138	0 0	—	—	139	0 0	—	—
2803	" Supreme	124	0 0	129	0 0	141	1 0.7	158	0 0
2808	" Goldfinder	138	0 0	135	0 0	188	0 0	159	0 0
2495	<i>A. nuda</i> , Hull-less	151	0 0	—	—	155	0 0	—	—

5-foot drills at the rate of 200 grains per row. The intensity of the attack of smut was estimated at maturity by counting the healthy and infected panicles in each row. The results are summarised in Table IV.

The difference between the two races of smut was very striking, especially in the series which included shelled grain. *U. avenae* (L 11), isolated from *A. strigosa*, infected only the three sub-species of *A. strigosa* together with *A. brevis*. *A. nuda* and twenty-six varieties of *A. sativa* remained immune. The intensity of the attack varied from 66 to 93 per cent. *U. avenae* (L 12), isolated from *A. sativa*, Potato, failed to infect *A. nuda*, *A. brevis* and *A. strigosa*, but thirteen varieties of *A. sativa* were attacked, the degree of infection ranging from 2 to 88 per cent.

These results include the highest infection figures yet obtained in Wales under field conditions, and it is interesting to find that the grain was subject to meteorological conditions which have proved to be favourable for infection of oats by smut. March 1926 was exceptionally dry with a total rainfall of only 1.16 in. The rain which fell during the interval, 2 days before to 5 days after sowing, amounted only to 0.177 in. The average temperature at a depth of 4 in. in the soil at 9 a.m. daily was 42.9° F. The data are of interest in connection with Fig. 1.

Table V.

*Showing the behaviour of four spore collections of U. avenae on twelve varieties of oats. 1927. Reference C 166.*

Species and variety of host	Percentage infected plants produced by spore collections of <i>U. avenae</i>			
	L 1, Wales	L 12, Wales	L 2, U.S.A.	L 11, Wales
	ex <i>A. sativa</i>	ex <i>A. sativa</i>		ex <i>A. strigosa</i>
Season 1927. Germinated in sand—				
<i>A. sativa</i> , Sandy (1799)	100	90	100	0
„ Potato (2855)	100	100	100	0
„ Radnorshire sprig (1103)	80	30	82	0
„ Victor (2394)	90	40	100	0
„ Abundance (2807)	0	0	100	0
„ Record (2804)	10	30	100	0
„ Black Tartar (2806)	90	20	100	0
„ Markton (2375)	0	0	0	0
<i>A. nuda</i> (2495)	0	0	100	0
<i>A. strigosa orcadensis</i> (521)	0	0	30	67
<i>A. strigosa glabrescens</i> (2877)	0	0	0	30
<i>A. brevis</i> (2384)	0	0	0	10
Season 1927. Germinated on filter-paper—				
<i>A. sativa</i> , Potato (2855)	100	100	100	0
<i>A. nuda</i> (2495)	0	—	100	—
<i>A. strigosa orcadensis</i> (521)	—	0	—	100

(b) *Germination in sand under controlled conditions of temperature and moisture.* 1927.

The same races of *U. avenae*, L 11 and L 12, were tested again in 1927 on twelve varieties of oats by the method of germination in sand (p. 70). Two additional samples of *U. avenae*, L 1 and L 2, were also included for comparison. The results are summarised in Table V.

*U. avenae* (L 11) again infected only varieties of *A. brevis* and *A. strigosa*. *U. avenae* (L 2, Missouri) infected *A. nuda* and a number of *sativa* varieties heavily and produced some infection on *A. strigosa orcadensis*. Collections L 1 and L 12 probably represent the same biological species. They infected certain *sativa* varieties but gave negative results on *A. nuda*, *A. brevis* and *A. strigosa*, and by these characters are distinguished from L 2 and L 11. It is evident that at least three biological species of *U. avenae* are represented by these collections<sup>1</sup>.

2. *U. levis* (C 3) isolated from *A. sativa*, Grey Winter in England.

In 1925, by the kindness of Dr G. Pethybridge, two collections of *U. levis* were received from Hertfordshire, the one (C 3) on a *sativa* variety Grey Winter, the other (C 4) on an unknown variety. Infection experiments conducted with each collection indicate that both represent the same biological species.

Table VI shows the results obtained in 1926 and in 1927 with four spore collections of *U. levis*. The tests were carried out by method C.

*U. levis* (C 1) produced infection only on varieties of *A. strigosa* and *A. brevis*, while *U. levis* (C 2) attacked varieties of *A. strigosa*, *A. nuda* and *A. sativa*. These results were expected from previous experience with the collections.

Collections C 3 and C 4 produced an intense attack on three *sativa* varieties, including the original host Grey Winter, but they gave low or completely negative results on *A. nuda* and *A. strigosa*, and on this character appear to be distinct from the Missouri strain C 2. This result was obtained in three separate experiments.

Before leaving the question of biological specialisation it is worthy of record that the *sativa* variety Markton, which remained immune in all trials conducted in the U.S.A., gave negative results in Wales with each of the seven biological species of *U. avenae* and *U. levis* which have been examined (Tables V and VI).

<sup>1</sup> It is not impossible that a collection like L 2, which produces infection on varieties belonging to widely different species of the host, may be a mixture of two or more biological species.

Table VI.

*Showing the behaviour of four spore collections of U. levis on seven varieties of oats. 1926-27. Reference C 164.*

Species and variety of host	Percentage infected plants produced by four spore-collections of <i>U. levis</i>			
	C 1, Wales	C 2, U.S.A.	C 3, England	C 4, England
Season 1926. Germinated in sand—				
<i>A. strigosa orcadensis</i> (521)	100	100	9	0
<i>A. brevis</i> (2384)	100	0	0	0
<i>A. nuda</i> (2495)	0	95	25	26
<i>A. sativa</i> , Potato (2855)	0	100	100	100
" Victor (2394)	0	100	62	80
" Grey Winter (2860)	0	95	96	96
" Markton (2791)	0	0	0	0
Season 1927. Germinated in sand—				
<i>A. strigosa orcadensis</i> (521)	—	100	0	5
<i>A. nuda</i> (2495)	—	100	20	15
<i>A. sativa</i> , Grey Winter (2860)	—	100	100	90
Season 1927. Germinated on filter-paper—				
<i>A. strigosa orcadensis</i> (521)	—	100	—	0
<i>A. nuda</i> (2495)	—	100	—	28
<i>A. sativa</i> , Grey Winter (2860)	—	86	—	100

#### IV. THE RELATIVE RESISTANCE OF LINE SELECTIONS OF *AVENA STRIGOSA* TO *U. LEVIS*, BIOLOGICAL SPECIES C 1.

In a previous paper reference was made to the varied behaviour of different lots of *A. strigosa* in regard to infection by covered smut (13). Data collected from published papers and from experiments carried out in Wales showed that certain lots were immune while others were markedly susceptible to the same race of smut (*loc. cit.* Table II).

During the seasons 1925-27, 101 line selections of *A. strigosa* were tested for resistance to the biological species of *U. levis* C 1. The data are summarised in Table VII. The most severe attack of smut was obtained in 1926 from grain sown in the open on March 31st. The heavy infection was probably due to similar conditions of rainfall and temperature which operated in experiment C 166 (Table IV). With shelled grain forty-eight lines suffered an attack of over 90 per cent. and in seventeen lines every plant of both rows was infected. The grouping of the lines in Table VII is based on these results.

Selections possessing extreme susceptibility occurred in each of the three sub-species of *Avena strigosa*. Of the resistant lines which gave under 10 per cent. infection with shelled grain in 1926, two belonged to



the sub-species *orcadensis*, twenty-seven to the sub-species *glabrescens*. Among the latter, nine remained immune in three seasons' tests.

It is evident that *A. strigosa*, in common with other species of *Avena*, includes varieties which vary within the widest possible limits in their reaction to smut fungi<sup>1</sup>.

In any study of biological specialisation it is important that referenced pure lines should be employed as hosts for the parasites under investigation.

Table VII.

*Showing a summary of infection experiments with pure lines of Avena strigosa and Ustilago levis, biological species C 1. 1925-27.*

Sub-species and variety of <i>A. strigosa</i>	Ref. to group	Degree of infection 1926, shelled grain	No. of samples 1925	Grain in husk smutted tillers %	No. of samples 1926	Grain in husk smutted tillers %	Shelled grain smutted tillers %	No. of samples 1927	Shelled grain smutted plants %
<i>Glabrescens</i> —									
var. <i>cambrica</i>	I	Nil	9	0	10	0	0	10	0
" "	II	Under 10 %	18	0.4	19	1.3	2.2	19	0.1
" "	III	Over 10 %	20	6.3	18	41.6	75.2	5	39.2
var. <i>albida</i>	III	Over 10 %	1	13.0	1	46.0	93.0	1	24.1
<i>Pilosa</i> —									
var. <i>fusca</i>	III	Over 10 %	7	7.4	7	50.7	95.1	5	39.4
var. <i>alba</i>	III	Over 10 %	1	0	1	3.8	38.0	1	18.9
<i>Orcadensis</i> —									
var. <i>intermedia</i>	II	Under 10 %	2	0	2	1.7	6.4	2	0
" "	III	Over 10 %	35	13.4	37	55.8	93.5	4	16.8
var. <i>flava</i>	III	Over 10 %	6	15.9	6	53.8	91.9	4	23.5

#### V. OBSERVATIONS ON THE NATURAL SMUT INFECTION OF CERTAIN BRITISH OAT VARIETIES.

Data concerning the incidence of smut in commercial samples of oats grown at the Station during the seasons 1919 to 1922 were given in previous papers (16, 15). Among varieties most heavily infected are Potato and its allies, Ceirch du bach, Radnorshire Sprig and Tyrone Tawny.

Since 1923 a narrower range of varieties has been grown at the Station. Those named above continued to carry from time to time relatively severe attacks of loose smut, and in addition to these the following varieties yielded a considerable number of smutted panicles: *A. strigosa glabrescens* and *orcadensis*; *A. sativa*, Record, Grey Winter, Black Winter,

<sup>1</sup> The data are not sufficiently complete for publication, but evidence is available that selections of *A. strigosa* vary also in their resistance to loose smut (*U. avenae* L 11).

Black Bell and Lund. The species of smut on *sativa* varieties was invariably *U. avenae*.

A numerical estimation in 1926 of the attack of smut in a crop of Radnorshire sprig, described as "considerably smutted," showed that the infection amounted to  $6 \pm 0.1$  per cent. Records exist from other parts of the country of cases of smut attack which were estimated at 50 and 75 per cent. (7, 8), but these are probably of rare occurrence in the British Isles.

In 1927, 225 samples of oats were obtained from various districts of Britain and Ireland. They were sown in single (55 samples) or in duplicate (170 samples) rod rows at the farm on April 13th. Infected panicles were cut from each row as they made their appearance and the number taken was duly recorded for each sample. The species was in all cases *U. avenae*. Rows which produced more than one smutted panicle were ultimately harvested and the total number of fertile tillers was counted. The grain had been sown thickly and the panicles per row varied from 200 to 1000. The data are summarised in Table VIII. Nineteen samples were omitted as foreign, mixed, or not true to name.

The general intensity of the attack was slight. Only 5 samples produced more than 10 per cent. of smutted panicles. These belonged to either the Winter, Potato, or Sprig groups, the infection ranging from 11 to 33 per cent. (Table IX). Excluding 3 samples of Marvellous, all the samples (44) which yielded more than 1 per cent. of smut were representatives of the same three groups of varieties.

The trial included 55 samples of the newer *sativa* varieties of the group *verna* IV, and 70 samples of the sub-species *orientalis*. Considering these together 104 samples were entirely free from smut and 18 showed less than 1 per cent. infection. The data as a whole are in close agreement with those published by Stapledon (16).

The freedom from smut of the variety Black Tartar here and in previous trials is of interest in view of the fact that this variety showed marked susceptibility under experimental conditions (Table V). It is tentatively suggested that this and other varieties may be constitutionally susceptible, but liable to escape heavy infection by their method of flowering. Preliminary observations made in 1926 showed that some varieties rarely or never exerted their stigmas, while others, growing under the same conditions, opened their pales widely and exposed the stigmas fully. This habit would facilitate the contamination of the flower by spores of loose smut. The 5 samples which gave over 10 per cent. infection, 2 which were only slightly attacked and 1 which remained free from smut, served as material for a microscopic examination designed

Table VIII.

Showing the incidence of smutted panicles in 206 samples of oat varieties from the British Isles. 1927. Reference C 183.

Reference to group of varieties*	Number of samples in which smutted panicles occurred to the extent of					Total no. of samples
	0 %	Under 1 %	1-5 %	5-10 %	Over 10 %	
<i>Avenae sativa</i> —						
Sub-species <i>autumnalis</i>	9	1	4	4	2	20
„ <i>verna</i> (Group I)	1	2	5	0	2	10
„ <i>verna</i> (Group II)	9	13	12	1	1	36
„ <i>verna</i> (Group IV)	55	8	0	0	0	63
„ <i>verna</i> (Group V)	2	2	0	0	0	4
„ <i>orientalis</i>	57	10	3	0	0	70
<i>Avena strigosa</i>	2	1	0	0	0	3

\* The groups included the following varieties. The figures in brackets indicate the number of samples of each variety.

*A. sativa autumnalis*. Bountiful (7), Black Winter (10), Grey Winter (3).

*A. sativa verna*. Group I. Radnorshire sprig (5), Sparable (1), Tyrone Tawny (1), Ceirch du bach (3).

Group II. Potato (16), Sandy (6), Tam Finlay (3), Long Houghton (1), Challenge (1), White Cluster (1), Castleton (6), Blainslie (2).

Group IV. Abundance (21), Victory (23), New Market (2), Svalof King (1), Record (2), Crown (4), Fortune (2), Ascott (1), Beaseler (1), Ligowo (1), King (3), White Horse (1), Banner (1).

Group V. Poland (1), Yellow Giant (1), Golden Rain (2).

*A. sativa orientalis*. Black Tartar (20), White Tartar (1), Superb (4), Supreme (10), Yields (6), Harvester (2), Cropwell (1), Earl Haig (1), Marvellous (25).

to discover the resting form of the fungus on the grain. Twenty grains from each of 8 samples were examined by the method referred to above (p. 73). The results are shown in Table IX.

Chlamydospores, solitary or in clusters, were seen occasionally, but they were not so frequent or so abundant as a certain slender, branched and frequently budding mycelium which sometimes covered the inner surface of one or both pales (Plate IX, fig. 2 (a) and (b)). A coarser type of mycelium, often dark in appearance, occurred not infrequently, but it was usually easy to distinguish it from the more slender type, which stained particularly well with cotton blue. A comparison was made with that observed on the pales of grain which had been contaminated artificially with spores during the flowering stage. There seems little doubt that the slender type represents the resting form of *U. avenae* such as Zade(19) described. This identification is supported by the results quoted

in Table IX, since the number of grains carrying the mycelium was highest in samples which yielded the greatest number of smutted panicles in the field.

These data serve to emphasise the fact that the incidence of smut in any particular crop of oats is determined not only by the degree of infection in the parent crop but also by the climatic conditions during the period of flowering, since these will influence both the number and the germination of spores which are carried between the pales.

Table IX.

*Showing the results of a microscopic examination of 20 grains taken from each of 8 samples included in experiment C 183 (Table VIII). Five of the samples produced over 10 per cent. of smutted panicles under field conditions in 1927.*

Variety of <i>A. sativa</i>	Station number	District from which sample was obtained	Smutted panicles %	Grains showing gemmae and mycelium on the pales %
Sandy	3174	Durham	33	50
Bountiful	3319	Somerset	19	40
Ceirch du bach	3247	Pembrokeshire	15	35
Bountiful	3306	Devon	13	15
Radnorshire sprig	3298	Hereford	11	58
Sandy	3224	Wigtonshire	4	10
"	3137	Belfast	2	0*
"	3255	Montrose	0	0

\* Chlamydospores were present on the inner pale of one grain, but none had germinated.

## VI. SUMMARY.

1. Various methods of testing varieties of oats for resistance to smut are described. A technique which involves the germination of shelled grain in sand of low moisture content at a temperature of 22° C. gave the most satisfactory results.

2. Data are given of the infection capacities of four biological species of *U. avenae* and three of *U. levis*. Two of these are described for the first time.

3. Pure line selections of *A. strigosa* vary in their resistance to *U. levis* (C 1) from 0 to 100 per cent.

4. A large number of commercial samples of oats was examined for smut infection. The attack was heaviest on varieties belonging to the Winter, Potato or Sprig groups. Samples yielding over 10 per cent. of smut were examined microscopically and typical resting mycelium of *Ustilago avenae* was found abundantly on the pales.

## VII. ACKNOWLEDGMENTS.

The author desires to thank Dr G. Pethybridge, Pathological Laboratories, Harpenden, and Dr G. M. Reed, Brooklyn Botanic Gardens, New York, for material of oats infected by smut.

Grateful acknowledgment is made to Dr Eastham, National Institute of Agricultural Botany, Cambridge, who supplied 100 samples of oats, and to the following individuals and firms for assistance in procuring further samples: Mr T. Anderson, Board of Agriculture for Scotland; Mr I. W. Seaton, Plant Breeding Division, Belfast; Mr David Thomas, Agricultural Organiser, Bulth Wells; Messrs James Carter and Co., Raynes Park, London; Mr H. H. Dunn, Salisbury; Messrs Gartons, Ltd., Warrington; Mr Hartley, Aberystwyth; Messrs Leighton, Ltd., Newcastle, Staffordshire; Messrs McGill and Smith, Ltd., Ayr, N.B.; Messrs Temperley and Co., Newcastle-upon-Tyne; Messrs Toogood and Sons, Southampton; Messrs Vilmorin-Andrieux et Cie, Paris; Messrs Edward Webb and Sons, Stourbridge.

The author is greatly indebted to Mr M. G. Jones, M.Sc., for samples of his own pure line selections of *Avenae strigosa*, and to Mr E. T. Jones, B.Sc., for most valuable help in connection with the classification of oat varieties. Special mention must be made of the detailed care given by Mr Watkins to the cultures described in the above paper.

Sincere thanks are given to Professor R. G. Stapledon for making available the facilities of the Welsh Plant Breeding Station, without which it would have been impossible to carry out the work, and for his constant interest and help.

## VIII. REFERENCES.

- (1) ARLAND, A. (1924). Der Haferflugbrand, *Ustilago avenae* (Pers.) Jens. Biologische Untersuchungen mit besonderer Berücksichtigung der Infektions- und Anfälligkeitsfrage. *Botan. Archiv*, vii, 70-111.
- (2) BARTHOLOMEW, L. K. and JONES, E. S. (1923). Relation of certain soil factors to the infection of oats by loose smut. *Journ. Agric. Res.* xxiv, 569-575.
- (3) BRIGGS, F. N. (1927). Dehulling barley seed with sulphuric acid to induce infection with covered smut. *Journ. Agric. Res.* xxxv, 907-914.
- (4) DIEHL, O. (1925). Experimentelle Untersuchungen über die Lebensweise und Bekämpfung des Haferflugbrandes. *Botan. Archiv*, xi, 146-199.
- (5) JOHNSTON, C. O. (1927). Effects of soil moisture and temperature and of dehulling on the infection of oats by loose and covered smuts. *Phytopath.* xvii, 31-36.
- (6) MARQUAND, C. V. B. (1922). Varieties of oats in cultivation. *Welsh Plant Breeding Station Bulletin*, Series C, No. 2, 44 pp.
- (7) MINISTRY OF AGRICULTURE AND FISHERIES (1922). *Fungus diseases of crops 1920-21*, p. 16.
- (8) — (1926). *Fungus and allied diseases of crops 1922-24*, p. 14.



Fig. 1

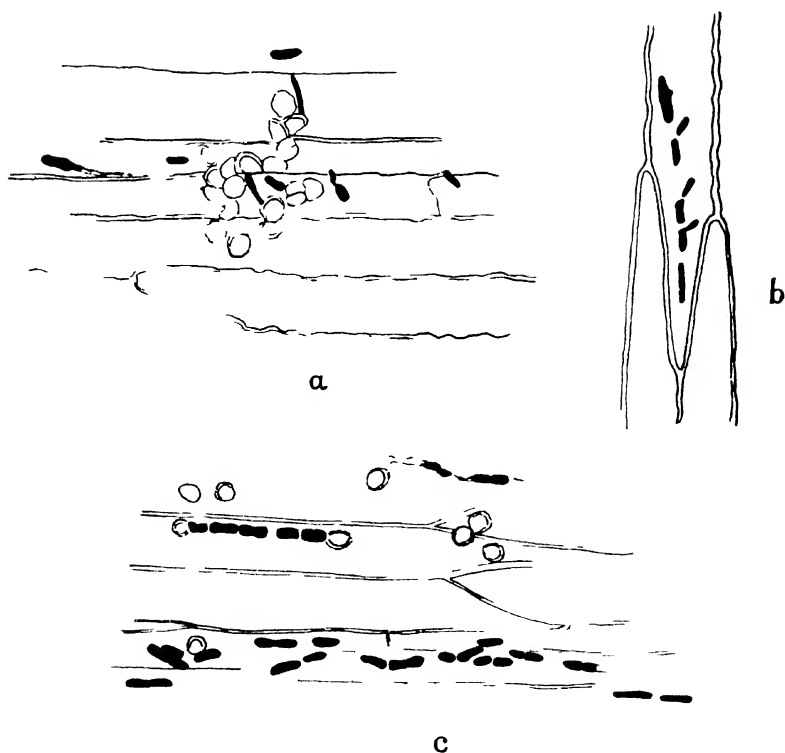


Fig. 2

SAMPSON.—THE BIOLOGY OF OAT SMUTS (pp. 65-85).



- (9) REED, G. M. (1924). Physiologic races of oat smuts. *Amer. Journ. Bot.* xi, 483-492.
- (10) — (1927). Further evidence of physiologic races of oat smuts. *Mycologia*, xix, 21-28.
- (11) REED, G. M. and FARIS, J. A. (1924). Influence of environmental factors on the infection of sorghums and oats by smuts. *Amer. Journ. Bot.* xi, 502-512, 579-599.
- (12) ROSCH, A. (1926). Studien über den Haferflugbrand, *Ustilago avenae* (Pers.) Jens. und den Glatthaferbrand, *Ustilago perennans* Rostr., mit besonderer Berücksichtigung der Immunitätsfrage beim Haferflugbrand. *Botan. Archiv*, xiii, 384-431.
- (13) SAMPSON, KATHLEEN (1925). Some infection experiments with loose and covered smuts of oats which indicate the existence in them of biological species. *Ann. App. Biol.* xii, 314-325.
- (14) — The biology of oat smuts. I. Viability of chlamydospores. *Ann. App. Biol.* xvi, 586-612.
- (15) SAMPSON, KATHLEEN and DAVIES, D. W. (1923). Incidence of fungus diseases on oat varieties in the seasons 1921-22. *Welsh Plant Breeding Station Bulletin*, Series C, No. 3, 55-57.
- (16) STAPLEDON, R. G. (1921). Variety trials with oats. *Welsh Plant Breeding Station Bulletin*, Series C, No. 1, 28-31.
- (17) TISDALE, W. H. (1923). An effective method of inoculating barley with covered smut. *Phytopath.* xiii, 551-554.
- (18) TRÆEN, A. E. (1925). Über den Einfluss der Temperatur und der Feuchtigkeit auf den Brandbefall des Hafers durch gedeckten Haferbrand (*Ustilago laevis* (K. & S.) Mag.). *Meldinger fra Norges Landbruks høiskole*, v, 157-168.
- (19) ZADE (1922). Experimentelle Untersuchungen über die Infektion des Hafers durch den Haferflugbrand (*U. avenae* Jens.). *Fühlings Landw. Zeit.* Lxxi, 393-406.
- (20) — (1924). Neuere Untersuchungen über die Lebensweise und Bekämpfung des Haferflugbrandes (*Ustilago avenae* [Pers.] Jens.). *Angew. Bot.* vi, 113-125. *Rev. Appl. Myc.* iii, 642, 1924.

## EXPLANATION OF PLATE IX

Fig. 1. Showing the behaviour of four biological species of smut on *Avena strigosa orcadensis* (Cc 2062). Infection obtained by method (C). 1925.

- (a) *U. levis* (C 1) ex Wales. 100 per cent. infection.
- (b) *U. levis* (C 2) ex U.S.A. 80 per cent. infection.
- (c) *U. avenae* (L 1) ex Wales. 0 per cent. infection.
- (d) *U. avenae* (L 2) ex U.S.A. 50 per cent. infection.

Fig. 2. Oat pales carrying chlamydospores and mycelia identified as *Ustilago avenae*.

- (a) Group of chlamydospores on the inner pale of the variety Sandy (Cc 3174 in Table IX). Natural contamination.
- (b) Budding mycelium from another part of the same pale.
- (c) Chlamydospores germinating on the surface of the inner pale of Potato oat (Table III, series C). Artificial contamination of exerted stigmas.

The drawings were made by the aid of a camera lucida under a 4 mm. objective and a 10 × eyepiece. × 490.

(Received May 9th, 1928.)



## THE CONTROL OF "BUNT" IN WHEAT

By W. A. R. DILLON WESTON, M.A.

*(School of Agriculture, Cambridge.)*

THE purpose of the following paper is to discuss the efficacy of the methods of treatment employed in the control of bunt in wheat.

RESULTS OF CONTROL MEASURES AGAINST "BUNT" IN WHEAT CARRIED OUT ON EXPERIMENTAL PLOTS AT THE CAMBRIDGE UNIVERSITY FARM.

1. *A comparison between bluestone, formaldehyde and copper carbonate.*

It is the custom to arrange each year a series of demonstration plots showing methods of controlling this disease. These trials have included a comparison of the formaldehyde and bluestone methods of treatment; and, in the past two seasons, a comparison between the above methods and the so-called dust or powder treatment which consists in treating the grain in a dry way with a powder such as copper carbonate. The percentage of bunt is estimated at harvest by taking a count of 1000 heads in a diagonal band. The size of the plots is not standardised, but in any one year the plots are uniform in size. In 1924 each plot consisted of approximately 300 ft. of drill, in 1925 of 200 ft. and in 1926 of 100 ft.

The land generally available for these experiments is a poor light soil, and normally not one which would be seeded for wheat.

The wheat used is "Little Joss," and in every case prior to sowing this is very heavily artificially contaminated with bunt spores. When this wheat is sown in these experimental plots it is literally black, and no farmer normally would sow such wheat.

It is sometimes considered that this variety is more susceptible to bunt than other varieties, and, in order to determine if this is correct, twelve wheats, as follows, were contaminated at the rate of one part of bunt spores to twenty-five parts of wheat and sown under conditions as uniform as possible in field experiments. At harvest, Wilhelmina showed 73 per cent. of bunted ears, Rector 55 per cent., Benefactor 84 per cent., Squareheads-Master 68 per cent., Victor 71 per cent., Marshal Foch 67 per cent., Iron 69 per cent., April Bearded 89 per cent., Yeoman 82 per cent., Little Joss 72 per cent., Rivett 78 per cent., Red Marvel 80 per cent.

These figures suggest that Little Joss is not more susceptible than other English varieties.

Table I.

*Result of four years' trials of bunt control.*

Treatment and method	Year	Germination	% of bunted ears
Untreated	1921-22	100	55
	1923-24	98	63
	1924-25	94	87
	1925-26	99	90
Formaldehyde (1 : 240) 1 pint to 30 gallons. Treated by steeping method	1921-22	100	0.6
	1923-24	98	0.2
	1924-25	81	0.3
	1925-26	97	0.0
Copper sulphate 2½%. Treated by steeping method	1921-22	93	7
	1923-24	91	3
	1924-25	78	6
	1925-26	90	2
Copper carbonate 3 oz. per bushel. Treated by the dusting method	1924-25	97	15
	1925-26	99	10

The following figures show that the best method with heavily contaminated seed is the formaldehyde treatment. In interpreting these figures, however, it must be remembered that the wheat used had previously been artificially contaminated at an extremely high rate, as witness the percentage of bunt in the untreated plots. Such grain would not be used by the farmer for his seed wheat.

It is of interest to note that germination has only once been materially affected by the formaldehyde treatment, whereas in every case the use of copper sulphate has caused an appreciable reduction. The low germination with the formaldehyde and copper sulphate in 1924-25 was due very largely to the sample being one which was badly threshed, many grains having been broken. It will be seen that the dusting method, using copper carbonate, has not affected the germination. It must be noticed, however, that an infection of 87 and 90 has only been reduced by this treatment to 14 and 10 respectively, whereas the formaldehyde and copper sulphate treatments have reduced it considerably more than this.

## 2. *Concentration at which to use formaldehyde.*

At what concentration is it most economical to use formaldehyde?

Salmon has shown previously<sup>1</sup> that formaldehyde diluted 1 : 320 (1 pint to 40 gallons of water) was as effective in controlling bunt as the 1 : 240 solution (1 pint to 30 gallons of water). At a later date<sup>2</sup> Salmon used

<sup>1</sup> *Journal of the Ministry of Agriculture*, xxvii, 1921, 1013.

<sup>2</sup> *Journal of the Ministry of Agriculture*, xxix, No. 8, Nov. 1922.

formaldehyde solutions at the following dilutions: 1 pint of formaldehyde to respectively 40, 60, 80 and 100 gallons of water. The results obtained showed that the formaldehyde became less efficacious the more it was diluted below the 1 : 480 (1 pint to 60 gallons) limit, but that at this concentration it gave a perfectly satisfactory control of bunt.

In the season 1923-24 formaldehyde was used at three concentrations: 1 : 240, 1 : 480 and 1 : 640. Both the steeping and sprinkling methods were employed. Table II shows the results of control measures carried out against bunt in wheat for the season 1923-24 when 40 per cent. formaldehyde was used at different concentrations. From these figures it is clear that there is a wide range over which formaldehyde is both safe and effective. The strength now recommended in the Ministry of Agriculture's leaflet No. 92 is 1 pint of 40 per cent. formaldehyde to 40 gallons of water (*i.e.* a concentration of 1 : 320). This gives a safe and economical method of controlling the disease.

Table II.

*Results of bunt control trials with formaldehyde of different strengths.*

Material	Treatment		Result	
	Strength	Method of application	Germination	% of bunted ears
40 Formaldehyde	1 : 240 (1 pint to 30 gallons)	Sprinkled*	98	0.6
40 "	1 : 240 (1 pint to 30 gallons)	Steeped	99	0.4
40 "	1 : 240 (1 pint to 30 gallons)	Sprinkled*	98	0.2
40 "	1 : 240 (1 pint to 30 gallons)	Steeped	97	0.0
40 "	1 : 480 (1 pint to 60 gallons)	Sprinkled*	99	0.2
40 "	1 : 480 (1 pint to 60 gallons)	Steeped	99	0.4
40 "	1 : 640 (1 pint to 80 gallons)	Sprinkled*	97	0.2
40 "	1 : 640 (1 pint to 80 gallons)	Steeped	99	0.5
Untreated	—	—	98	63

\* Sprinkled at rate of 1 gallon to 2 bushels.

### 3. *Dusting method.*

This method was first applied by Darnell-Smith in Australia. In the United States of America it is stated that copper sulphate represses root growth, and formaldehyde the development of the young shoot. It was on this account that Darnell-Smith's method was adopted in that country.

In order to test the value of dusts such as copper carbonate in controlling the disease, trials have been carried out during the past two seasons<sup>1</sup>. Various so-called "copper dusts" have been used which included copper carbonate, copper acetate, copper sulphate, copper stearate and copper arsenite.

<sup>1</sup> *I.e.* 1924-5, 1925-6.

In the season 1925-26 the copper carbonate used was drawn from a 2-ton consignment to Australia, shipped mainly for the purpose of seed wheat dressing.

Little Joss wheat was treated with varying amounts of crushed "bunt balls," sown, and the percentage of bunted ears estimated at harvest by taking a count of 1000 ears from a diagonal band across each plot, these consisting of six rows, each 16 ft. long.

Table III shows the percentage of bunted ears from untreated and treated samples, previously contaminated at different rates with crushed "bunt balls." It is clear from these figures that infection varies directly with the spore load and that the efficacy of the dusting treatment is proportional to the rate of contamination.

In the same season three plots were laid down in which contaminated wheat was dusted with varying amounts of copper carbonate (*i.e.* 3, 6, 9 oz. to the bushel). These results, showing the effect of an increased dressing of copper carbonate, are analysed in Table IV.

Table III.

*The percentage of bunt in treated and untreated wheat contaminated at different rates.*

Rate of treatment with bunt	Treatment	% of bunted ears
1 to 25	Copper carbonate 3 oz. per bushel	11
1 to 25	Untreated	94
1 to 50	Copper carbonate 3 oz. per bushel	5
1 to 50	Untreated	78
1 to 100	Copper carbonate 3 oz. per bushel	3
1 to 100	Untreated	50
1 to 500	Copper carbonate 3 oz. per bushel	1
1 to 500	Untreated	25
1 to 25	Untreated	92

Table IV.

*Showing the effect that an increased dressing of copper carbonate has in reducing the percentage of bunted ears.*

Treatment	Rate	Germination	% of bunted ears
Copper carbonate	3 oz. per bushel	99	24
" "	6 oz. per bushel	99	14
" "	9 oz. per bushel	99	6
Untreated	—	99	86

*Practical aspect of dusting wheat.* For the application of this treatment a dusting machine is required. There are several of these on the American, Australian and German markets. Of these the "Calkins" wheat-treating machine was used by the writer during the season 1926-27, and was found to work efficiently. The makers state that 30 bushels per hour can be treated with this machine, but, working experimentally, we were not able to reach this standard.

If no such dusting machine is available a suitable contrivance can easily be arranged from an old barrel or churn, or more simply by rolling a barrel containing the grain and powder. There is, however, one serious objection to this treatment, and that is the danger of inhaling the fine copper powder. This danger may be overcome by the labourer wearing a mask, or it can be minimised by carrying out the dusting operation in the open air. Using small hand machines, or home-made contrivances, the danger to the workman by this method may be considerable. Given, however, a good machine—that is, one which is dust proof—the operation should involve no danger—or even necessitate the wearing of a mask. Although it is not suggested that at the present this method should supplant the use of formaldehyde or bluestone, it is considered that this treatment is efficacious when dealing with large bulks of slightly contaminated wheat.

#### RELATIVE COSTS FOR THE THREE TREATMENTS WITH FORMALIN, COPPER SULPHATE AND COPPER CARBONATE.

Costs based on treatment of seed for two days' sowing: viz. ten sacks  
-acreage sown, sixteen acres.

##### *Copper sulphate treatment by the steeping method.*

	s.	d.
2 man hours     ...     ...     ...     ...     ...     ...	1	4
2 boy hours     ...     ...     ...     ...     ...     ...	0	8
Material (2½ per cent. sol. copper sulphate)		
(10 lbs. copper sulphate at 4d. per lb. to 40 gallons		
of water)     ...     ...     ...     ...     ...     ...	3	4
	<u>5</u>	<u>4</u>

Approximate cost per acre, 4d.

*Formalin treatment by the steeping method.*

	s.	d.
2 man hours	1	4
2 boy hours	0	8
Material (1 pint 40 per cent. formalin at 1s. 9d. per pint to 40 gallons of water)	1	9
	<u>3</u>	<u>9</u>

Approximate cost per acre, 3d.

*Copper carbonate treatment by the dusting method, using a machine<sup>1</sup>.*

	s.	d.
4 men hours at 8d.	2	8
2 engine hours	2	0
2 oz. copper carbonate per bush. (5 lb. at 1s. 4d. per lb.)	6	8
	<u>11</u>	<u>4</u>

Approximate cost per acre, 8d.

With reference to the price of copper carbonate a firm of Commercial Chemists, —, states:

When we sell in more or less large quantities for export to Australia, we charge 8½d. per lb. for 5-ton lots in casks and 9d. per lb. if in kegs; for a ton or less we charge 9½d. per lb., and quite small quantities anything from 10d. to 1s. per lb. It also depends upon whether we sell to merchants or to actual users, as of course merchants have to make something out of the business themselves. If your enquiry refers to a price that farmers in this country should pay, we suggest about 1s. 3d. per lb. for anything below 1 cwt., and about 1s. per lb. for 1 cwt. and over.

Although the cost of treatment by the dusting method is at least twice that of the others, it must be borne in mind that the great advantage of the dusting treatment is in its convenience, enabling as it does seedsmen to send out stocks already treated. It is difficult to convert this very appreciable advantage into terms of money.

*Feeding treated wheat to poultry.* Although it is inadvisable to feed poultry with dressed wheat, it is desirable to ascertain whether injury to poultry will result from feeding seed wheat so dressed. Feeding trials on dressed wheat were accordingly carried out by the Poultry Nutrition Section of the Animal Nutrition Institute, Cambridge, 4 oz. of dressed wheat being fed daily per bird to four White Leghorn hens over a period

<sup>1</sup> Depreciation on machine not taken into account.

of 5 days. The trials showed that, for short periods at least, such dressed wheat can be safely fed to birds. The samples of wheat fed had been dressed as follows:

Sample 1. Wheat steeped for 2 minutes in  $2\frac{1}{2}$  per cent. copper sulphate solution.

Sample 2. Wheat steeped for 2 minutes in formaldehyde, strength 1 : 320.

Sample 3. Wheat treated with copper carbonate powder 3 oz. per bushel.

Analyses by Mr H. G. Pike showed that the dressed wheat contained 0.016 per cent.  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  and 0.101 per cent. copper carbonate respectively.

In concluding, the writer wishes to express his thanks to the following who have assisted him: Mr F. R. Petherbridge, with whom the writer co-operated in the 1923-24 experiments, Mr A. Amos for permission to reproduce the results of experiments in 1921-22, and for the figures relating to the costs of the three treatments, Mr E. T. Halnan for his report on feeding of "dressed" wheat to poultry, and Dr G. H. Pethybridge and Dr H. Hunter for constructive criticisms.

#### SUMMARY.

1. Results of control experiments over a period of four years are given. The wet treatment, with formaldehyde and copper sulphate, are compared with the dry treatment, using copper carbonate.

2. The dusting method is discussed. It is shown that infection varies directly with the spore load and that the efficiency of the dusting treatment is proportional to the rate of contamination. For the usually slightly contaminated seed samples this treatment has been effective in controlling bunt.

3. The practical aspect of dusting wheat is discussed and its cost compared with the other treatments.

4. A report is given of the feeding of dressed wheat to poultry.

*(Received July 23rd, 1928.)*

# THE ACTION OF SULPHUR AS A FUNGICIDE AND AS AN ACARICIDE

## PART II

BY WM. GOODWIN AND H. MARTIN.

(Research Department, South-Eastern Agricultural College, Wye, Kent.)

(With one Text-figure.)

FROM the examination by chemical methods of the mechanism of the action of sulphur "at a distance" which formed the subject of our previous communication<sup>(3)</sup> certain conclusions were drawn which it was proposed to test by biological means. The main inference was that the agent concerned in this action, and which is formed when sulphur is applied to a heated surface, is elementary sulphur generated by volatilisation. This conclusion was based upon the fact that the agent, although reduced in amount by passage through a glass-wool filter maintained at ordinary temperatures was unaffected by filtration through a glass-wool plug heated to the temperature of the original sulphured surface.

The biological indicators employed were firstly, the powdery mildew of the hop (*Sphaerotheca humuli* DC., Burr.), chosen because it had been stated by Salmon<sup>(5)</sup> that under ordinary conditions actual contact of the sulphur particles with the fungus appears to be necessary for the fungicidal action to take place. Young leaves bearing vigorously growing patches of the "powdery" conidial stage of the fungus were selected for treatment, the other leaf at the same node being employed for the control experiment<sup>1</sup>. Secondly, the mildew *Erysiphe graminis* DC., growing upon couch (*Agropyron repens* Beauv.) was used, the couch being transplanted into pots prior to use. This fungus was chosen as the nearest approach available to the form growing upon young wheat plants, which was the one employed by Barker, Gimingham and Wiltshire<sup>(1)</sup>. Only vigorously growing leaves bearing several densely powdery patches were selected for treatment, the control being a leaf as far as possible similar in growth. Thirdly, the black currant gall mite (*Eriophyes ribis* (Westw.) Nal.) was included as it was the organism concerned in Lee's experiments<sup>(4)</sup>, and evidently was extremely susceptible to the action of sulphur

<sup>1</sup> The plants used were selected by Professor Salmon, who also kindly examined the fungus after treatment.



at a distance. It was found that twigs bearing big buds could be cut from the parent bush and kept in water without interference to the mites to an extent that would seriously affect the experiments.

It is not proposed to give in detail preliminary experiments designed for the determination of the optimum conditions of experiment, but to enter at once upon the discussion of the trials in their ultimate form noting, where necessary, the points observed in the preliminary work.

(a) ACTION AS A FUNGICIDE.

The apparatus finally evolved is represented in Fig. 1. For the majority of the experiments recorded below the long tube contained a plug of glass-wool dusted with sulphur at (a). Below were two further plugs of clean glass-wool (b) and (c). The tube was thus divided into three portions, each of which was jacketed and the air (drawn from outside the laboratory) was blown first through the sulphured plug and then into the lower tube (d). The leaf, still attached to the parent plant, was placed within the tube (d) and was held in position by a copper clip. To protect the leaf tissue, a paper band (e) was gummed round the inside of the lower edge of this tube. For the control the apparatus was identical in every respect, except for the absence of sulphur or for a different treatment of the middle jacket; the leaves and also the patches of fungus were chosen as nearly as possible similar to those used in the experiment. The passage of the air through each apparatus was maintained at equal rates, whilst by a careful regulation of the heating it was possible to equalise the temperatures within the lower tube (d) just above the leaf surface. Strict control of this temperature, determined by a thermometer inserted at (f) was essential, for it was found that the use of temperatures much above 30° C. caused a collapse of the conidiophores, a condition which could not be prevented by increasing the humidity of the air. The air was in all cases initially saturated with moisture by passage through washing bulbs which also permitted the adjustment of the air speed.

From preliminary work it was found that even prolonged exposure had little if any immediate effect upon the powdery mildew of the hop. Thus in a series of experiments in which a composite glass-wool plug consisting of a wad of sulphur-dusted glass-wool supported by a wad of clean glass-wool to prevent the passage of actual sulphur particles removed by the current of air, was placed at (c), the control tube containing a glass-wool plug without sulphur, and the jackets heated by steam yielded the following results:

*Sphaerotheca Humuli.*

*Exp.* 27/13. Exposed 3 hours at average temperature in sulphur tube 24.9° C. (max. 25.5° C.), control tube 25.5° C. (max. 26.5° C.).

Examination with a hand lens showed that although on the second day after treatment some slight difference may have existed between the fungus patches of the treated and control leaves, by the fourth day the fungus was growing well on both leaves.

*Exp.* 27/14. Exposed 4 hours, average temperature in sulphur tube 27.2° C. (max. 28° C.), in control tube 28.7° C. (max. 31° C.).

Lens examination failed to show any difference in the health of the fungus upon the treated and control leaves. That the sulphur volatilised in the sulphur tube was reaching the surface of the leaf was established by placing thereon a small piece of clean copper foil. The foil became slightly tarnished in 30 minutes and was completely blackened within 90 minutes of the start of the treatment.

*Exp.* 27/15. Exposed 9 hours, average temperature in sulphur tube 27.7° C. (max. 29.5° C.), in control tube 29.1° C. (max. 30° C.).

Examination with lens on the first and fourth days after treatment showed no difference between treated and control leaves, upon both of which the fungus was apparently unaffected.

Preliminary trials similar in character made upon *Erysiphe graminis* gave strong indications of an action:

*Erysiphe graminis.*

*Exp.* 27/20. Exposed 4 hours, average temperature in sulphur tube 27.5° C. (max. 28.5° C.), in control tube 27.2° C. (max. 28° C.).

The fungus on the treated leaf showed immediate signs of change and a lens examination after 24 hours showed that all except one conidiophore upon the treated area had collapsed. Within 3 days a patch on the treated area showed a fresh growth of conidiophores. The fungus on the control leaf was apparently unaffected.

*Exp.* 27/21. Exposed 4 hours, average temperature in sulphur tube 26.4° C. (max. 29° C.), in control tube 26.4° C. (max. 29° C.).

Lens examination after 24 hours showed that the conidiophores upon the treated area of the leaf in the sulphur tube had collapsed and were of an ochraceous brown colour, but the radiating mycelium was still white; after 5 days certain of the mildew patches were again growing vigorously and glistening with conidiophores. The fungus on the control leaf was apparently unaffected.

Such results were definite indications that *E. graminis* was more rapidly affected by the sulphur volatilised from the glass-wool than *S. Humuli*, and the experiments carried out with the apparatus (A) shown in Fig. 1 were aimed not only at testing the removal of the volatile agent by its condensation and removal by the cooled glass-wool plug but also at establishing the existence of this specific action of the two fungi towards sulphur. Accordingly, after each apparatus had been heated till conditions were uniform and steam was passing from the exit tubes of

the lower jackets, the hop leaf, across which was placed the couch leaf, was fixed in position. In the second apparatus (B) containing similar plugs the middle jacket was cooled with cold water. The lower steam jacket enabled the maintenance of equal temperatures in the two tubes.

The following were the results obtained:

*Exp. 28/42.* Time of exposure  $6\frac{1}{2}$  hours, average temperature in tube A (all jackets steam heated)  $26.5^{\circ}\text{C}$ . (max.  $29.5^{\circ}\text{C}$ .), in tube B (middle jacket cooled)  $27.3^{\circ}\text{C}$ . (max.  $28^{\circ}\text{C}$ .).

Lens examination, after 24 hours, showed no signs of fungicidal action nor of differences in the fungi upon the leaves in tubes A and B.

*Exp. 28/43.* Tubes A and B as in *Exp. 28/42*, but no couch leaves employed. Exposed for  $6\frac{1}{2}$  hours, average temperature in tube A  $22.3^{\circ}\text{C}$ . (max.  $23.5^{\circ}\text{C}$ .), in tube B  $22.5^{\circ}\text{C}$ . (max.  $24^{\circ}\text{C}$ .).

After 24 hours there was no apparent difference between the two leaves and the fungus was apparently unaffected.

As in these two experiments it was found that even after several days no signs of fungicidal action developed, the leaf was, in subsequent experiments, removed from the parent plant after treatment and examined under the microscope, a procedure not adopted in the above experiments, since it was thought that indications of fungicidal action might be slow in appearing.

*Exp. 28/44.* As in *Exp. 28/43*, couch leaves placed over the hop leaves. Exposed for  $5\frac{1}{2}$  hours, average temperature in tube A  $28.1^{\circ}\text{C}$ . (max.  $30^{\circ}\text{C}$ .), in tube B  $27.0^{\circ}\text{C}$ . (max.  $28.5^{\circ}\text{C}$ .).

An examination with a lens immediately after withdrawal from the tubes showed no difference nor sign of action upon any of the treated leaves. After 24 hours, microscopic examination showed that with *S. Humuli* the fungus upon the leaf from tube A only showed a few shrivelled conidiophores—99 per cent. were healthy and upright; no signs of action were observed upon the leaf from tube B. With *E. graminis*, the leaf exposed in tube A showed many collapsed and shrivelled conidiophores, especially at the edges of the leaf, though certain areas bore healthy conidiophores. No signs of action were apparent upon the mildew patches from tube B. The copper clip used to retain the leaves in position was at the conclusion of the exposure markedly tarnished in tube A, that in tube B showing but the slight dullness to be expected after exposure to air.

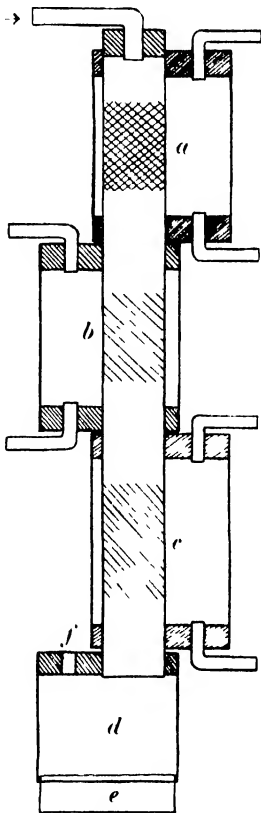


Fig. 1.

*Exp. 28/45.* As in the above, time of exposure 7 hours, average temperature of tube A 28.5° C. (max. 30° C.), of tube B 27.8° C. (max. 29° C.).

Microscopic examination revealed that on both the hop and couch leaves treated under tube A the fungus was affected, the chains of conidia were shrivelled to the extent of 50 per cent. or over. Of the leaves treated under tube B the fungus upon the couch leaf showed no signs of fungicidal action, whereas in the case of *S. Humuli* although there were certain conidiophores showing spores either partly or wholly shrivelled, their number did not approach 50 per cent.

*Exp. 28/46.* As in the above, time of exposure 6 hours, average temperature in tube A 29.5° C. (max. 31.5° C.), in tube B 29.0° C. (max. 31° C.).

The copper clip under tube A had developed a reddish stain 2 hours after placing in position. Microscopic examination showed that on both the hop leaves the fungus was affected in certain areas. Upon the couch leaves, that under tube B was apparently unaffected; whilst upon the leaf from tube A one patch of mildew showed many shrivelled conidiophores, an adjacent patch was apparently healthy.

*Exp. 28/47.* As in the above, time of exposure 9½ hours, average temperature in tube A 28.7° C. (max. 31° C.), in tube B 28.7° C. (max. 31° C.).

Upon all the areas treated in both tubes A and B there were but slight signs of fungicidal action—occasional conidia were shrivelled. It was found, however, that even on an untreated leaf of the hop the fungus had a similar appearance.

These last six experiments, which were the concluding ones of a long series, show well the failure to secure conclusive results when employing fungi as the test material. This inconclusiveness is due primarily to the slowness of the action of sulphur upon the fungus, a point to which we must return later. A further important factor was the failure to secure controls completely healthy, a failure to be attributed mainly to two facts, firstly, the rapid fungicidal action of hot air, secondly, the shrivelling of the conidia under normal conditions. It was thought that possibly the action of the hot air might depend upon the desiccation of the conidia, but trials in which the treated leaf was placed upon filter paper kept continually moist still showed shrivelled conidia at the end of the exposure. As regards the normal shrivelling of the conidia the nature of the experiment prevented a preliminary microscopic examination of the fungus, and so the existence of shrivelled conidiophores prior to treatment could not be established except by reference to untreated patches of the mildew. Yet another difficulty was introduced into the microscopic examination of the fungus after treatment owing to the effects of the treatment being localised to a remarkable extent. In many cases it was found that although at one point the fungus would be completely shrivelled and collapsed, an adjacent area would show little sign of fungicidal action. It was therefore necessary to examine the mildew patches at many points before arriving at a final conclusion which was of necessity frequently

indefinite in character. This curious localised action it is suggested may be due to a failure of the air to penetrate the densely packed conidiophores, for it was often observed that signs of action were confined to the edges of the mildew patch. If such be the case it would be analogous to the condition found in the use of fungicidal sprays, where without the addition of a spreader the patches could not be uniformly wetted (2).

Although the experiments must be considered to have failed in their initial objects, (1) to show the removal by filtration through a cooled wad of glass-wool of the volatile agent formed when sulphur is heated, (2) to establish the specificity of the fungi *S. Humuli* and *E. graminis* towards the action of sulphur, they throw considerable light upon the mode of action of sulphur as a fungicide. It was evident that the amount of sulphur volatilised when heated to almost 100° C. for 8 to 9 hours was insufficient to cause a complete collapse of the conidiophores of the fungi. In view therefore of the enormous reduction of the vapour pressure of sulphur with temperature the vaporisation of the sulphur could play only a small part in the exertion of its fungicidal action at ordinary temperatures. Strong support is therefore given to the view that actual contact of the fungus with the sulphur particle is necessary before it is killed. In many cases the microscopic examination revealed apparently healthy and turgid conidia bearing many small particles of sulphur, an indication that in the action of sulphur at a distance the re-condensation of the volatilised sulphur upon the fungus is part of the mechanism of the fungicidal action and that sulphur vapour is not directly toxic to the fungus.

These opinions were therefore tested by experiments in which the leaf bearing many vigorously growing mildew patches was placed inside a glass tube coated on the interior with sulphur. The plant was placed in an unheated glasshouse and the temperature within the tube recorded by means of a thermometer.

*Exp. 27/42.* One hop leaf was enclosed in a sulphured tube, whilst the opposite leaf at the same node was dusted with sulphur and placed in a similar tube. The leaves were exposed from 8. vi. 27 to 12. vi. 27; the temperatures being read at 9 a.m., 12 noon, 3, 6 and 9 p.m. yielded an average of 21° C. (70° F.) with a maximum of 33° C. (91.5° F.). Although the patches of mildew dusted with sulphur showed, at the end of treatment, the characteristic fungicidal action of sulphur, those upon the leaf in the sulphured tube were apparently unaffected.

*Exp. 27/43.* Three tubes, two of which were arranged as in *Exp. 27/42*, a third containing a leaf upon which "colloidal" sulphur had been painted around a vigorously growing patch of *S. Humuli*, were set up. The leaves were exposed from 13. vi. 27 to 16. vi. 27; the temperatures read at the same intervals as in *Exp. 27/42* yielded an

average of 24.7° C. (76.5° F.) with a high maximum of 40° C. (104° F.). Examination on the 19th, three days after the extremely hot day, showed that on the control leaf the tips of certain conidiophores were withered. Whilst the fungus dusted with sulphur was showing the typical signs of fungicidal action no difference could be observed between the control and the fungus on the leaf placed in the sulphured tube nor even between the control and the mildew patch surrounded by "colloidal" sulphur.

*Exp. 28/53.* Six tubes were arranged as in the above experiments, three containing couch leaves bearing vigorously growing patches of *E. graminis*, three containing hop leaves bearing patches of *S. Humuli*. One of each set served as control, whilst the other two tubes were painted on the inside with sulphur. The plants were placed in an unheated glasshouse from April 5th to May 21st (46 days). Upon removal, all the mildew patches were found to be growing vigorously despite the presence of a parasitic fungus which had itself developed in the presence of the sulphur.

These results definitely indicate that the production of volatile sulphur at ordinary temperatures is insufficient to bring about the toxic action known to be possessed by sulphur against these two fungi.

#### (b) ACTION AS AN ACARICIDE.

Preliminary experiments indicated that the black currant gall mite is extremely sensitive to sulphur and that the organism is affected even by the small amount of sulphur volatilised at ordinary temperatures. The following are the results of a series of trials carried out with an apparatus similar to that employed for experiments 27/13-15 recorded in section (a). In the sulphur tube was placed a composite glass-wool plug consisting of glass-wool dusted with sulphur supported by a clean glass-wool wad, the control tube contained a clean glass-wool plug, whilst the outer jacket of both tubes was heated by steam. Twigs bearing big buds from which the mites were actively emerging were placed in the lower tubes, the bud being adjacent to the bulb of the thermometer.

*Exp. 28/81.* Buds exposed 1 hour, temperature in both tubes 17.5° C.

Examination under a binocular microscope showed that, whereas the mites upon the control bud were moving vigorously, the majority of those on the bud exposed in the sulphur tube were motionless and apparently dead.

*Exp. 28/82.* Buds exposed 40 minutes, temperature in both tubes 21.5° C.

Examination with the microscope 30 minutes after treatment showed no movement on the bud exposed in the sulphur tube, approximately 10 per cent. of the mites on the control bud were moving, a percentage which was markedly increased when examined an hour later.

It was evident that if movement was to be accepted as the criterion of non-acaricidal action, the buds after treatment should be exposed to conditions which would be favourable to the movement of any mites still alive. It was found that at low temperatures the mites would become

extremely sluggish and movement could in most cases only be detected in the slow waving of the legs. Such mites on exposure to sunlight or when placed near the steam oven again quickly became active. In the majority of the experiments recorded below counts of the moving mites were made after exposure for at least 90 minutes to sunlight or warmth. Owing to this delay in taking the counts it was found that a certain number of active mites were observed even on the treated buds, active probably because they had emerged from the bud subsequent to the treatment. As was to be expected, it was found that exposure of the bud to sulphur vapour did not affect the mites still hidden inside the bud and so protected.

Further, it was usually found that a small number of mites upon the control buds were motionless and in many cases obviously dead. Their death may have been due to desiccation or to natural mortality, for they were frequently observed even upon untreated buds.

The counts were carried out upon at least five different fields obtained by placing the bud in various positions under the microscope, the average percentage of mites moving being recorded below.

*Exp. 28/85.* Buds exposed for 6 hours, both tubes unheated and temperature at buds 13.5° C.

Examined after exposure to sunlight, 24 hours after the start of the treatment:

	Total no. mites examined	% mites moving
Control tube	95	85.3
Sulphur tube	61	6.6

*Exp. 28/95.* Buds exposed 3½ hours, both tubes unheated and temperature at buds 18.0° C.

Examined next day after exposure to sunlight:

	Total no. mites examined	% mites moving
Control tube	367	67.8
Sulphur tube	204	34.3

*Exp. 28/96.* Buds exposed 7 hours, tubes unheated and at average temperature 22.0° C.

When examined immediately after treatment it was found that the mites upon the control bud were apparently unaffected, the bud being densely packed with moving mites too numerous to count. Upon the bud from the sulphur tube, of the enormous number of mites observed, only three could be found showing signs of movement.

It is interesting to note that in the chemical part of this investigation it was found that clean copper foil was but slowly tarnished by exposure to air passed over sulphur heated at 38° to 40° C., the black currant gall

mite would appear to be a more sensitive reagent for the detection of sulphur vapour.

In view of this sensitivity of the mite towards sulphur vapour it was apparent that in using the three-jacketed apparatus (Fig. 1) a high mortality was to be expected, even in the control apparatus, where the greater part of the sulphur vaporised in the upper part of the tube was removed by the cooled glass-wool plug. The experiments, however, all showed a marked reduction in the percentage of mites affected by the passage of the air through the cooled filter:

*Exp. 28/90.* Buds exposed 3 hours, counts made after exposure for  $1\frac{1}{2}$  hours to sunlight:

	Tube A (all jackets heated)	Tube B (middle jacket cooled)
Average temp. of bud ° C.	15.1	15.0
Total no. mites observed	201	178
% mites moving	2.5	20.2

*Exp. 28/92.* Buds exposed  $1\frac{1}{2}$  hours, counts made 24 hours after treatment and after exposure for  $1\frac{1}{2}$  hours to sunlight:

	Tube A	Tube B
Average temp. of bud ° C.	17.1	16.5
Total no. mites observed	307	273
% mites moving	3.0	41.0

*Exp. 28/93.* Buds exposed  $1\frac{1}{2}$  hours, counts made 20 hours after treatment and after exposure for  $1\frac{1}{2}$  hours to sunlight:

	Tube A	Tube B
Average temp. of bud ° C.	18.7	18.8
Total no. mites observed	142	165
% mites moving	5.0	35.7

As a check, these buds were afterwards placed for 1 hour near the steam oven; recounts yielded the following results:

Total no. mites observed	334	208
% mites moving	6.9	43.3

*Exp. 28/94.* Buds exposed 1 hour. A control trial in which the bud was left untreated was included in this experiment which gave the following results:

	Tube A	Tube B	Control
Average temp. of bud ° C.	19.5	19.5	16.0
Total no. mites observed	187	194	201
% mites moving	12.8	36.6	83.6

From these experiments it is permissible to conclude that the agent responsible for the death of the gall mite and which is evolved when sulphur is heated is gaseous sulphur. Its amount is diminished by passage through a cooled glass-wool filter, though evidently this condensation



## 102     *Sulphur as a Fungicide and as an Acaricide*

and filtration process still permits the passage of sufficient volatile sulphur to produce a marked effect upon the gall mite.

As it has been suggested that sulphur dioxide or hydrogen sulphide are the agents formed from heated sulphur and responsible for its action at a distance, experiments were carried out to determine the action of these gases upon the gall mite. Similar trials proved unsatisfactory in the case of the fungi owing to the direct action of these gases upon the leaf and the possibility that their action upon the fungus was therefore indirect.

For the purpose of these experiments the air was first passed, at a rate of approximately 500 c.c. per minute, through a dilute solution of sulphuric acid to which could be added by means of a drop funnel, in the one case, measured amounts of a 10 per cent. solution of crystalline sodium sulphite,  $\text{Na}_2\text{SO}_3 \cdot 7\text{H}_2\text{O}$ , in the other case, measured amounts of a solution of crystalline sodium sulphide,  $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ . These solutions were freshly prepared for each experiment.

Trials in which the bud was held for 1 minute and for 10 minutes in an atmosphere smelling strongly of sulphur dioxide and in which moist blue litmus paper was immediately reddened showed that the mites, although perhaps at first affected, rapidly recovered on exposure to sunlight. In one experiment the bud was exposed for  $4\frac{1}{2}$  hours during which time 0.051 gm. sulphur dioxide passed through the apparatus (average concentration approx. 0.013 per cent. by volume sulphur dioxide). Examination of the bud after 24 hours showed no difference between the mites of the treated bud and those on a control bud exposed to a similar stream of air drawn from outside the laboratory. Even in a trial in which the bud was exposed, at  $18^\circ \text{C}$ ., for 5 hours to an average concentration of 0.029 per cent. by volume sulphur dioxide, no apparent effect upon the mites was observed though a small leaf adjacent to the bud was wilted after the treatment.

Similarly, experiment showed that hydrogen sulphide at such concentrations was without permanent effect upon the mite. After an exposure, at  $20^\circ \text{C}$ ., for 90 minutes to a concentration of 0.208 per cent. by volume hydrogen sulphide followed by exposure to sunlight for 1 hour, the mites were found to be less active upon the treated bud. No difference, however, could be detected between the mites and those of a control bud after exposure to sunlight for 3 hours.

## SUMMARY.

The conclusion arrived at by chemical methods, that the volatile agent produced when sulphur is applied to a heated surface is gaseous sulphur, has been subjected to biological tests in which the fungi *Erysiphe graminis* and *Sphaerotheca Humuli* and the gall mite *Eriophyes ribis* were employed.

The fungi were found not to be sufficiently sensitive to yield satisfactory and concordant results and strong support is given to the view that actual contact of the sulphur particle with the fungus is necessary before fungicidal action can occur.

It was shown that the agent present in air passed over heated sulphur and responsible for the death of the gall mite was not removed by filtration through a heated glass-wool plug, this observation being contrary to the view that the toxic agent is produced initially in solid form.

Filtration through a cooled glass-wool plug only removed part of the volatile agent and it was shown that the gall mite is affected by the traces of sulphur volatilised at ordinary temperatures.

The results of the experiments with the gall mite were in complete accord with those obtained in the previous chemical work.

At relatively large concentrations sulphur dioxide and hydrogen sulphide are without permanent effect upon the gall mite and these gases are therefore not responsible for the acaricidal action of sulphur.

## REFERENCES.

- (1) BARKER, B. T. P., GIMINGHAM, C. T. and WILTSHIRE, S. P. (1919). *Long Ashton Ann. Rpt.* p. 87.
- (2) EYRE, J. V. and SALMON, E. S. (1916). *Journ. Agric. Sci.* VII, 473.
- (3) GOODWIN, W. and MARTIN, H. (1928). *Ann. App. Biol.* XV, 623.
- (4) LEES, A. H. (1923). *Journ. Pom. Hort. Sci.* III, 103.
- (5) SALMON, E. S. (1921). *Journ. Min. Agric.* XXVIII, 150.

(Received August 16th, 1928.)

# ON THE OCCURRENCE OF THE PARTHENO- GENETIC AND SEXUAL FORMS IN *APHIS RUMICIS* L., WITH SPECIAL REFERENCE TO THE INFLUENCE OF ENVIRONMENTAL FACTORS

BY J. DAVIDSON, D.Sc.<sup>1</sup>

(*Rothamsted Experimental Station, Harpenden.*)

(With 6 Text-figures.)

## CONTENTS.

	PAGE
I. INTRODUCTION . . . . .	104
II. METHODS OF BEARING THE VARIOUS PARTHENOGENETIC LINES . . . . .	105
III. NORMAL OCCURRENCE OF THE PARTHENOGENETIC AND SEXUAL FORMS . . . . .	108
IV. NORMAL OCCURRENCE OF THE PARTHENOGENETIC ALATAE AND APTERAET . . . . .	108
V. THE FUNDATRIGENIAE GENERATIONS . . . . .	109
(a) Offspring of the fundatrices . . . . .	109
(b) Offspring of the fundatrigeniae apterae . . . . .	110
(c) Offspring of the fundatrigeniae alatae (migrantes) . . . . .	111
VI. THE ALIENICOLAE GENERATIONS . . . . .	111
A. Occurrence of alatae and apterae . . . . .	112
(1) Offspring of the alienicolae apterae . . . . .	112
(2) Offspring of the alienicolae alatae . . . . .	113
(3) The effect of overcrowding on the occurrence of alatae and apterae . . . . .	113
(4) The effect of nutrition on occurrence of alatae and apterae . . . . .	116
(5) The effect of temperature on occurrence of alatae and apterae . . . . .	118
B. Occurrence of the sexual forms . . . . .	120
VII. GENERAL CONCLUSIONS AND SUMMARY . . . . .	127
REFERENCES . . . . .	134

## I. INTRODUCTION.

IN an earlier paper<sup>(5)</sup> the writer gave a short review of the more recent literature dealing with the occurrence of alate and apterous parthenogenetic females in aphides. Since that time several investigators have brought forward experimental evidence showing the influence of external factors on the occurrence of these forms, notably Brittain<sup>(3)</sup>, Mason<sup>(17)</sup>,

<sup>1</sup> Now at the Waite Agricultural Research Institute, University of Adelaide, South Australia.

Wadley(23), Ewing(11), Ackerman(1) and Reinhard(19). In most cases these writers have briefly summarised the views of previous observers so that it is not necessary to do so here.

The older views regarding the influence of environmental factors on the occurrence of the sexuales in aphides were also briefly discussed in my earlier paper(5). Little experimental work has been done on this problem, although it is apparent from field observations on several species that external factors play a large part in bringing about the cyclical change in these insects. Since Klodnitzki's (1912) extensive studies, which were dealt with in my previous paper, two further contributions to the literature are of particular interest. Uichanco(21) discusses the modifying influence of environmental factors on reproduction in the Aphididae and a later paper(22) contains an excellent account of the embryogeny and post-natal development of the Aphididae. Marcovitch(14,15) shows the importance of length of day as a factor affecting migration and the occurrence of the sexual forms in aphides.

In the present paper an account is given of the results obtained with *Aphis rumicis* L. (*A. fabae* Scop.) from rearing experiments carried on during the past 7½ years, particularly with reference to the two phenomena of the life-cycle referred to above.

The aims in view were (a) To trace the normal sequence of the generations in the complete life-cycle and the occurrence of the parthenogenetic and sexual phases. (b) To see whether the normal sequence could be affected experimentally by changing the environment in which the aphids were reared. (c) To observe the influence of environmental changes on the occurrence of alatae and apterae.

The experiments were commenced in June 1920 (Line A) with two apterous viviparous females taken from a wild colony on beans found in a local garden, and nine further related parthenogenetic lines were reared during succeeding years.

## II. METHODS OF REARING THE VARIOUS PARTHENOGENETIC LINES.

The methods employed in rearing the aphids have been already described(9). The relationships of the ten parthenogenetic lines are shown in Fig. 1 and Table I. In each of the eight lines, started with a Fundatrix, the aphids were reared on *Euonymus europaeus* until alate migrants developed (usually in 3rd generation), and afterwards on Longpod beans until the line was discontinued. In each year, about the end of August, colonies from the beans were established on *Euonymus* so as to ensure that sexuales would be obtained and fertilised eggs laid. When the

# 106 *Parthenogenetic and Sexual Forms in Aphis rumicis*

Table I.

Showing relationships of the 10 parthenogenetic lines of *A. rumicis*.

Line	Started with	Taken from line	Parthenogenetic generations		Ova	
			Started	Ended	Laid	Commenced to hatch
A	2 apterae	Wild colony	16 vi. 20	30. v. 21	Oct.-Nov. 1920	8. iii. 21
B	Fundatrices	A	8. iii. 21	31. xii. 23	Oct.-Nov. 1921	23. iii. 22
C	"	B	23. iii. 22	28. ii. 23	" 1922	None hatched
D	"	B	23. iii. 22	8. ii. 23	" 1923	17. iii. 24
E	"	B	17. iii. 24	30. xi. 24	Transfers not made for this purpose	
F	"	E	7. iv. 25	21. vi. 26	Oct.-Nov. 1924	7. iv. 25
G	"	F	24. iii. 26	30. xi. 26	" 1925	24. iii. 26
H	"	G	15. iii. 27	31. xii. 27	" 1926	15. iii. 27
Ha	Apterae	H	6. v. 27	31. xii. 27	" 1927	—
I	Fundatrices	G	20. iii. 27	9. xi. 27	" 1927	20. iii. 28
					" 1927	—

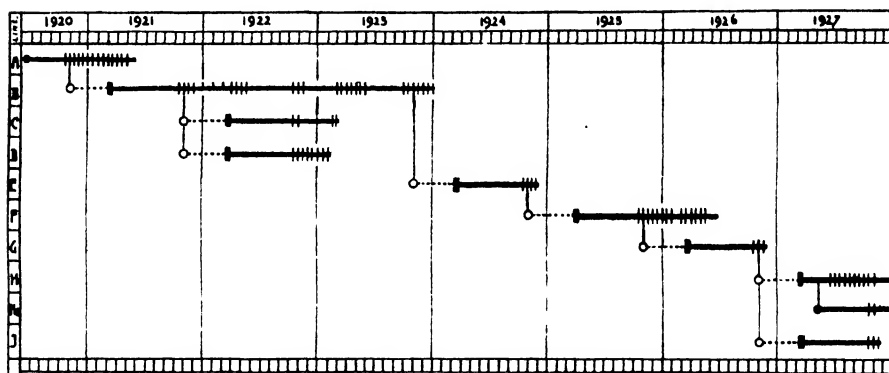


Fig. 1. Chart showing the relationships of the ten parthenogenetic lines of *Aphis rumicis* used in the experiments.

The years are divided into monthly periods. The broad black bands show the periods during which continuous parthenogenetic reproduction was maintained in each parthenogenetic line: the short cross-lines indicate the periods during which sexual forms occurred in the colonies: where the cross-lines are absent, only parthenogenetic individuals were obtained. Line B was started from one fertilised egg taken from line A and lines C-I are of the same strain, being related as shown with the thin connecting vertical lines.

With the exception of lines A and Ha each one was started from a fertilised egg.

○ = fertilised ovum.

■ = fundatrix.

● = apterous viviparous female.

During the winter period the aphids were reared under varying temperatures and with line H they received only 8 hours daylight daily from March to November. See also Figs. 4 and 5.

fundatrices began to hatch out the following spring, new lines were started as shown in Fig. 1.

Line A was started from a wild colony. Line B was started with one fundatrix from line A and since the subsequent lines were descended from line B, a single strain of the species has been used throughout. The aphids were reared in a large open glasshouse during the summer period, or in the open air insectary and in a heated glasshouse during the winter period. The air temperature at Rothamsted during the 7½ years is shown in Figs. 3 and 4 and also the temperature during the winter period in the heated glasshouse. The temperature in the summer glasshouse (1920–21) and open-air insectary (1927), during the summer period, is also shown. It will be seen that the temperature in the insectary approximates closely to that of the outside air temperature plotted from Rothamsted records and the temperature in the glasshouse during the summer period shows an average mean about 10° F. higher than the outside air temperature.

The *Euonymus* plants on which ova were laid in autumn, were kept outside in the open air during winter, being removed to the summer glasshouse in spring, soon after the fundatrices commenced to hatch out. By reference to Figs. 3 and 4 the conditions under which the aphids were reared can be seen from the following data: the symbols *O* = open air; *O.G.* = summer glasshouse; *H.G.* = heated winter glasshouse. It will be noted that the dates given for aphids in the open air (*O*) refer to living aphids; the ova from which the fundatrices were obtained were kept outside during winter.

*Line A.* 16. vi to 12. x. 20 (*O.G.*); 13. x. 20 to 31. iii. 21 (*H.G.*), 1. iv to 30. v. 21 (*O.G.*). *Line B.* 8. iii to 31. iii. 21 (*O*); 1. iv to 9. x. 21 (*O.G.*); 10. x. 21 to 20. iii. 22 (*H.G.*); 21. iii to 31. x. 22 (*O.G.*); 1. xi. 22 to 28. iii. 23 (*H.G.*); 29. iii to 19. x. 23 (*O.G.*); 20. x to 31. xii. 23 (*H.G.*). During the period 12. xi. 22 to 12. i. 23 the colonies received artificial light from electric lamps in addition to normal daylight (see Davidson, *Journ. Sci.* 1924, LIX, p. 364). A control series under the same temperatures received only normal daylight. *Line C.* 23. iii to 31. x. 22 (*O.G.*); 1. xi. 22 to 28. ii. 23 (*H.G.*): as in line B the colonies received artificial light, control colonies receiving only normal daylight. *Line D.* 23. iii to 31. x. 22 (*O.G.*); 1. xi. 22 to 8. ii. 23 (*H.G.*): the temperatures for this line, during the winter period, were lower than for B and C as can be seen in Fig. 3 (1927, middle line). *Line E.* 17. iii. to 30. xi. 24 (*O.G.*). *Line F.* 7. iv to 19. iv. 25 (*O*); 20. iv to 8. xi. 25 (*O.G.*); 9. xi. 25 to 20. iv. 26 (*H.G.*); 20. iv to 21. vi. 26 (*O.G.*). *Line G.* 24. iii to 30. xi. 26 (*O.G.*). *Line H.* 15. iii to 20. iv. 27 (*O.G.*); 21. iv to 30. ix. 27 (open-air insectary); 1. x to

## 108 *Parthenogenetic and Sexual Forms in Aphis rumicis*

31. xii. 27 (*H.G.*). From 28. iii to 5. ix. 27 the colonies in this line received only 8 hours daylight daily, being placed in a dark box from 5.30 p.m. until 9.30 a.m. daily. From 25. x to 23. xii. 27 the colonies were submitted to artificial light from electric lamps from sunset until 10 p.m. during 5 days each week: a control series reared under the same temperatures received only normal daylight, being kept in a dark box during the illumination period. *Line Ha.* 15. iii to 20. iv. 27 (*O.G.*); 21. iv to 30. ix. 27 (open-air insectary); 1. x to 31. xii. 27 (*H.G.*): this was a control line for line H, and after 5. v. 27 the aphids received normal daylight but, as in line H, a series of colonies received artificial light during the period stated and a control series had only normal daylight. *Line I.* 20. iii to 20. iv. 27 (*O.G.*); 21. iv to 9. xi. 27 (open-air insectary): this was a further control line for line H and the aphids received normal daylight.

### III. NORMAL OCCURRENCE OF THE PARTHENOGENETIC AND SEXUAL FORMS.

The normal life-cycle of the bean aphid as it occurs in England is shown graphically in the following diagram, together with the terms used in the present paper. A reference to this diagram will enable the reader to follow more readily the details discussed later.

### IV. NORMAL OCCURRENCE OF PARTHENOGENETIC ALATAE AND APTERAE.

It is clear from Fig. 2, that, if the normal bi-sexual cycle is to be completed, alatae must develop during two critical periods of the cycle, namely in spring when migration takes place from the winter host to the summer food-plants and again in autumn when the alate sexuparae (re-migrants) are due to return to the winter host plant. Furthermore, during the summer period, owing to the rapid reproduction of this species, it is necessary that alatae develop from time to time so as to prevent the starvation of the aphids in overcrowded colonies, and to ensure the distribution of the species to other food-plants. Since the sexual females (apterous) are produced by the alate sexuparae, it follows that, if experimental conditions are established such that a continuous line of apterous parthenogenetic females only are produced, the normal bi-sexual cycle cannot be completed. The occurrence of alatae therefore is closely associated with these three important features of the life-history and the environmental factors which influence migration and the change from the parthenogenetic to the sexual method of reproduction must also be considered in this respect as exercising an influence on the occurrence of

apterae and alatae. The alate form is the primitive condition and it seems to the writer, as already stated in a recent paper (10), that the logical interpretation of the problem of the occurrence of the alatae and apterous parthenogenetic females is, What factor or factors make for the occurrence of apterae? since by the continuation of a purely apterous parthenogenetic line, the distribution of the species is severely limited and the completion of the normal bi-sexual cycle is prevented.

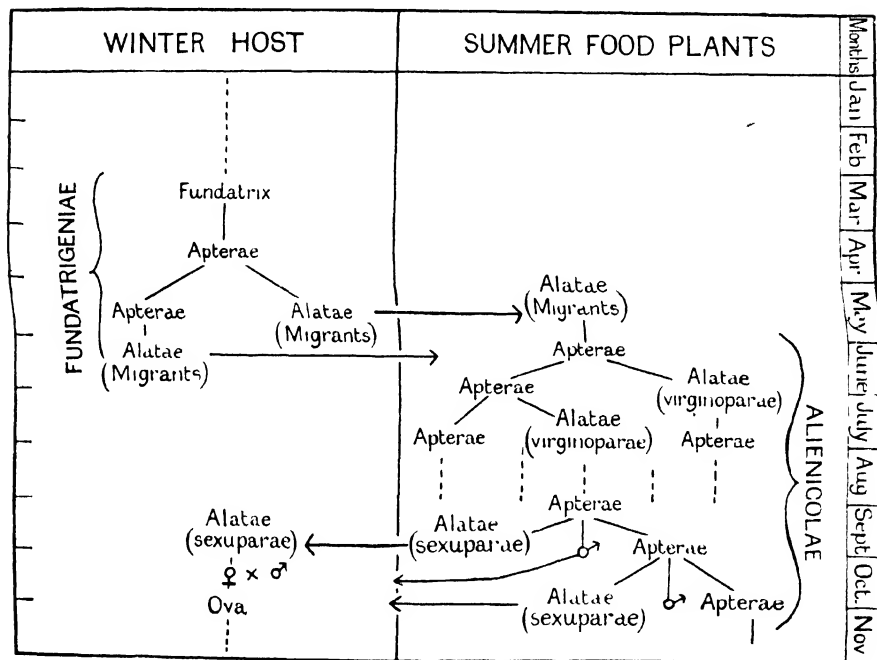


Fig. 2. Diagram illustrating the life-cycle of *Aphis rumicis*, showing the terms used for the different forms in the various generations.

#### V. THE FUNDATRIGENIAE GENERATIONS.

The fundatrigeniae are the descendants of the fundatrix, born on the winter host (Fig. 2) and consist of apterae and alatae, the latter being the migrants.

##### (a) Offspring of the fundatrices.

The offspring of the fundatrices are usually apterous but may consist of a mixed brood of apterae and alatae, the former being usually in the majority. The proportion of alatae which develop is affected by the condition of the food-plant (nutrition factor) and by the amount of young growth available in relation to the size of the colonies (overcrowding).



# 110 *Parthenogenetic and Sexual Forms in Aphis rumicis*

Counts made of the individuals present in four separate colonies on *Euonymus* produced by four fundatrices, after periods varying from 12 to 19 days, gave the following percentages of apterae—100, 83, 97, 11.5. The latter plant was "woody" and not making young growth, whereas on the other three plants there was plenty of young growth. Other instances were observed in which fundatrices produced a majority of alatae on "woody" *Euonymus* plants. When young shoots were maintained by cutting back the plants and overcrowding avoided, apterous fundatrigeniae produced a larger proportion of apterae in several successive generations. Fundatrices reared on Longpod beans and on *Rumex* produced freely and the offspring developed chiefly into apterae.

## (b) *Offspring of the fundatrigeniae apterae.*

The offspring of the apterae of 2nd generation on *Euonymus* develop chiefly into alatae (3rd generation) and although a few apterae may occur in this 3rd generation their offspring develop into alatae, so that after about three generations the spindle tree becomes free from the aphid, owing to this tendency for alate migrants only to develop. This sequence under natural conditions is correlated with overcrowding and nutrition, and the condition of the spindle tree does not favour the development of apterae when the flush of early spring growth is over. There is, however, apparently an inherent tendency for alate migrants to develop in these early generations on *Euonymus*, which is evidently associated with the evolution of the migrating habit. This tendency is affected by overcrowding and nutrition. Consequently, the proportion of alatae and apterae in a colony may vary considerably according to these conditions.

Table II.

*Showing offspring of fundatrigeniae apterae reared on Euonymus (1-7) and beans (8, 9).*

Colony no.	Date apterae transferred	No. transferred	Alatae and apterae in colony after 10 days		
			Days	Apterae	Alatae
1	25. iv. 14	2	25	0	Many
2	5. iv. 21	5	21	Few	"
3	5. iv. 21	6	21	"	"
4	30. iv. 22	1	19	0	58
5	25. iv. 22	1	21	1	44
6	30. iv. 22	1	19	1	55
7	9. v. 27	9	19	20	400
8	6. v. 27	2	18	19	2
9	6. v. 27	6	18	71	27

If they are favourable, apterae may be obtained in several successive generations.

In Table II (Nos. 1-7) examples are given showing the strong tendency for the offspring of apterae to develop into alatae when reared on *Euonymus*.

When reared on beans a higher proportion of apterae developed as shown in Nos. 8 and 9. Similarly on *Rumex* the proportion of apterae was greater. This appears to be due to the better nutrition afforded by these plants.

(c) *Offspring of the fundatrigeniae alatae (migrantes).*

The offspring of the migrantes are normally laid on intermediate food-plants and develop into apterae. When reared on *Euonymus*, on which plant they reluctantly reproduce, apterae develop if suitable young growth is available, but if the plant is "woody," alatae will also develop, which is further evidence of the unsuitability of *Euonymus* as a permanent food-plant and of the effect of nutrition on the occurrence of alatae. When successive generations, from the fundatrix, are reared on *Euonymus*, alatae tend to predominate (*vide* (5), p. 305)<sup>1</sup>.

## VI. THE ALIENICOLAE GENERATIONS.

The alienicolae generations are initiated by the alate migrants and we have seen that the unsuitability of the spindle tree as a summer food-plant and overcrowding are important factors affecting the progress of the rhythmical spring migration. The descendants of the offspring of alate migrants can be considered as alienicolae so long as an unbroken line of parthenogenetic generations is maintained. In line B, for instance, a line of alienicolae was carried on for  $2\frac{1}{2}$  years.

The following forms may develop in the alienicolae generations: (a) apterous viviparous females; (b) alate viviparous females (virginoparae) which produce parthenogenetic viviparous females; (c) alate viviparous females (sexuparae) which produce apterous sexual females; (d) alate males. The occurrence of these various forms is influenced by environmental factors, particularly length of day, overcrowding, nutrition and temperature. In nature, continuation of the parthenogenetic

<sup>1</sup> It is of interest to note in this respect that the autumn re-migrants (sexuparae) lay the sexual females below the old leaves of the spindle tree and the latter feed along the midrib and secondary veins, until they go to the branches in order to lay their eggs. The sexual females, therefore, are not dependent upon young growth. On the other hand, the spring migrants and also the alate virginoparae of the summer generations lay their offspring on or near the young growth of plants, on which the latter instinctively feed.

## 112 *Parthenogenetic and Sexual Forms in Aphis rumicis*

generations over the winter period is limited by seasonal and climatic factors. Under experimental conditions, however, favourable temperature and nutrition may be maintained and parthenogenetic reproduction carried on for long periods. Even under these conditions, the progress of the colonies during the winter period is slow compared with the summer period indicating the importance of the light factor<sup>1</sup>.

### A. OCCURRENCE OF ALATAE AND APTERAE.

As referred to above, the alatae may be virginoparae or sexuparae in addition to males. Actually in nature, in England, the two latter forms do not occur until late September, or early October, a period coincident with a falling mean temperature, decreasing hours of daylight and scarcity of suitable food-plants (poor nutrition). The alate virginoparae which develop during the summer period are to be considered as dispersal forms, whose function is to ensure the distribution of the species. Under experimental conditions these forms may develop during the winter period, as will be described later, although, with moderate temperatures the alatae produced during that period tend to be sexuparae. The occurrence of alate sexuparae in autumn marks the appearance of the sexual phase, and the environmental factors concerned, as we shall see later, are correlated with rhythmical seasonal changes. These factors are (a) length of day, (b) temperature, and (c) plant growth (nutrition) as affected by (a) and (b).

#### (1) *Offspring of the alienicolae apterae.*

The offspring of the apterae may develop entirely into apterae or alatae or consist of a mixed brood of apterae and alatae. The alatae may consist of virginoparae, sexuparae and males. The two latter occur when the sexual phase is in evidence and all the alatae at this time may be

<sup>1</sup> Several species have been reared over long periods in a continuous parthenogenetic line.

(1) Slingerland (1893), according to Uichanco (1921), reared *M. persicae* for 62 generations over a period of 2 years 10 months.

(2) Ewing (1916) reared *A. avenae* for 87 consecutive parthenogenetic generations in California.

(3) Paddock (1919) reared *A. gossypii* for 51 generations.

(4) Comstock (*Introduction to Entomology*, 1924, p. 417) states that Slingerland carried on a species for 98 generations over a period of 4 years and 3 months.

(5) Reinhard (1927) reared *A. gossypii* for 59 generations in Texas.

(6) The writer reared *A. rumicis* through 50 generations (line B) in a period of 2 years and 10 months at Rothamsted. The number of generations passed through in a given time depends upon temperature and whether the first- or last-born young are selected to carry on the next generation.

sexuparae and males. The former occur when conditions favour parthenogenetic reproduction, when all the alatae may be virginoparae. During October and November, under natural conditions, the offspring of the apterae may develop entirely into alate sexuparae and males thus bringing parthenogenetic reproduction to an end. Under experimental conditions, the alatae which develop during the autumn and winter period may consist of a mixed brood of both sexuparae and virginoparae, or only sexuparae and males or only virginoparae depending on environmental factors, particularly temperature. It was found, for instance, that when a temperature about a mean of 70° F. was maintained, apterae predominated and the few alatae which developed were virginoparae. No case was recorded in which apterae produced sexual females as was observed by Shull<sup>(20)</sup> with *Mac. solanifolii*. The proportion of apterae which develop as offspring of apterae is affected by overcrowding, temperature and nutrition (physiological condition of food-plant). As referred to earlier, length of day (light factor) is also important in that it influences the occurrence of the sexual phase.

(2) *Offspring of the alienicolae alatae.*

The alate virginoparae are usually produced by apterae as explained in the previous sections. Their offspring usually develop into apterae, but alatae may also develop if the conditions are unfavourable, as for instance, when alatae are compelled to reproduce on plants which are heavily infested with aphids (overcrowding), or with poor nutrition. The offspring of alate virginoparae are, however, not so variable in this respect as is the case with the offspring of apterae. The alate sexuparae always appeared in the colonies about the end of September (except in line H) and afterwards in many generations throughout the winter period. They produced only sexual females, and no case was recorded in which alate sexuparae produced males as recorded by Shull<sup>(20)</sup> with *Mac. solanifolii*. No case was observed in which alatae produced both sexual females and virginoparae.

(3) *The effect of overcrowding on the occurrence of alatae and apterae.*

It has been frequently observed throughout these experiments, especially during the summer period, that, when a colony is started with one or two apterae, only an occasional alate form develops during the first 14 days or so, but alatae gradually become dominant as the colony increases, so that by the time the plant is heavily infested, large numbers of alatae are present.

# 114 *Parthenogenetic and Sexual Forms in Aphis rumicis*

In order to test whether overcrowding was a factor affecting this increase in the number of alatae, 15 bean plants, one in each pot (seed planted on the same day), were divided into three series of five plants each. The plants were infected with adult apterae from line I on 3. vi. 27, all being of the same generation. In series A one individual was transferred to each plant, in series B, five individuals and in series C, ten individuals. The plants were kept covered with muslin bags and reproduction allowed to go on until 20. vi. 27, when the aphids were killed off and the number of apterae and alatae on each plant counted. The results obtained are shown in Table III.

Table III.

*Showing the effect of overcrowding on the occurrence of alatae.*

Series	Number of aphids per plant			Alate %
	Apterae	Alatae		
		Adult	Nymphs	
A	61	2	0	1
	59	0	0	
	52	0	0	
	45	1	0	
	31	0	0	
B	244	39	38	34
	208	41	45	
	179	90	46	
	177	33	24	
	165	80	71	
C	449	108	121	39
	363	133	89	
	358	48	46	
	284	216	116	
	228	101	107	

The column, *apterae*, includes adults and immature individuals obviously going to develop into apterae. The column, *alatae nymphs*, includes individuals which possessed wing pads. Younger individuals were not counted, as alatae and apterae cannot be readily distinguished in the earlier instars. Many of these younger individuals would of course develop into alatae, as is shown by the results of two other series of five plants each, which were set up at the same time. Each plant was infected with three apterae and reproduction was allowed to go on for 5 days longer than in the case of Table III. The counts of the colonies showed the percentage of alatae in the two series to be 77 and 81 respectively. The influence of overcrowding is shown by the number of *adult alatae* present. In series C (Table III) an overcrowded condition occurred

earlier in the colonies, resulting in alatae developing early, many being adult when the colonies were killed off. In the two series in which the colonies were started with three apterae, the average number of adult alatae in each colony was only fourteen, but the number of alatae nymphs, compared with series A-C, was considerably increased as the longer reproduction period allowed more of the later-born individuals, born under crowded conditions in the colony, to attain the 3rd and 4th instar stage in which the wing pads are visible. During the summer period, if overcrowding in the colonies is prevented, the offspring of the alienicolae apterae develop into apterae, but as soon as overcrowding occurs, alatae tend to predominate. In the parthenogenetic line C, a succession of colonies were maintained on beans from the beginning of May until September, overcrowding being prevented by removing the adults from time to time. The results obtained are given in Table IV.

Table IV.

*Showing offspring of apterae in successive colonies in the parthenogenetic line C when overcrowding was prevented.*

E = *Euonymus*; M = Migrants; S = Sexuparae; V = Virginoparae.

Colony (beans)	Aphids transferred (1922)	Alatae and apterae in colonies after 10 days		
		Days	Apterae	Alatae
1 (E)	17.4	19	25	4 (M)
2 (M)	5.5	17	25	0
3	19.5	9	51	0
4	25.5	11	50	0
5	6.6	12	3	0
6	18.6	16	Few	0
7	5.7	14	Many	0
8	19.7	12	Many	0
9	5.8	25	Few	Few (V)
10	21.8	21	Few	Few (S)
11	11.9	39	2	6 (S) + 6 ♂

Colony No. 1 was started with one fundatrix on *Euonymus* and No. 2 with two alate migrants from No. 1. The remaining colonies were started with one or two apterae from the previous colony. Apterae predominated until about the middle of August, from which time onwards the colonies developed more slowly and a comparatively small number of aphids was produced. This was due to the seasonal conditions, chiefly falling temperature and poorer growth of the bean plants, together with the advent of the sexual phase. Under these conditions, alatae tended to predominate

in the colonies and those which developed in September were sexuparae. It should be noted that, although alatae were not present in colonies 2 to 8 at the end of the period of days shown in column three, nymphs began to appear a few days later and, as overcrowding increased, the number of alatae increased.

It is clear that overcrowding is an important factor affecting the occurrence of alatae in the summer period when factors of light, temperature and nutrition (food plants) are favourable. The influence of overcrowding may to some extent be interpreted as a nutrition factor in that, the young growth of the plant being crowded, the aphids are forced to feed on the older tissues, and the sap of the young growth affords the best nutrition. Further, as the infestation increases, the tissues of the plant are so affected that they do not function in a normal manner and the quality and quantity of sap available is affected. Overcrowding is, however, relative and the phenomenon is not only a matter of nutrition, as overcrowding may occur in a comparatively small colony on a local area of a plant, resulting in an increase of alatae.

(4) *The effect of nutrition on occurrence of alatae and apterae.*

The writer has shown<sup>(4)</sup> that the reproduction rate of *A. rumicis* varies on different food-plants and is also affected by the physiological condition of the plant<sup>(8)</sup>. That the young growth of the bean plant affords the best nutrition for the insects is shown by the following experiment.

Two series, A and B, consisting of five bean plants each (seeds planted same day), were set up in pots. In series A the plants had normal growth, and in series B the young tops were cut off a few days before infection. On 31. v. 27 each plant in series A was infected with one adult apterous viviparous female from line I (offspring of alatae and reared to maturity on a normal bean plant). Similarly, each plant in series B was infected with one adult apterous viviparous female (offspring of alatae of same generation and reared to maturity on a bean plant having the top cut off). After 14 days' reproduction the ten plants were killed off and the aphids produced on each plant were counted. The results are shown in Table V.

It is evident that by removing the young top of the bean plant, its nutrition value for the aphid is affected. There is, however, no indication that the proportion of apterae and alatae has been influenced. It would be necessary to rear the aphids under these conditions through further generations to find out the cumulative effect, if any, of the two sets of

Table V.

*Showing the comparative reproduction rate on beans with normal growth (A) and with tops cut off (B).*

Series	Apterae	Alatae	Total aphids present	Mean
A	44	0	390	335.2
	34	0	276	
	33	0	384	
	32	0	339	
	29	5 (N)	287	
B	24	1 (N)	163	104.8
	24	0	165	
	23	0	84	
	14	0	72	
	10	0	40	

N = Nymphs.

conditions. Data available from experiments made to test the influence of different food plants and of the physiological condition of the bean plant on the reproduction rate of the bean aphid indicate that, on those plants which favour a high reproduction rate, the proportion of alatae which develop is smaller than on those plants on which a low reproduction rate occurs. This was observed, for instance, with poppies, peas, turnips and mangolds compared with broad beans, and its occurrence on *Euonymus* has been already referred to. Similar observations were made with different varieties of field beans. The available data does not allow of definite conclusions being drawn, but the factor of overcrowding does not appear to be the only one concerned, and the question as to whether the nutrition value of different food-plants affects the proportion of alatae and apterae which may develop requires further investigation. There is no doubt that the physiological condition of the food plant, in that it affects the nutrition of the insect, is a factor of importance, which must be considered when the influence of other external factors are being investigated if uniform results are to be obtained. The starvation experiments of Gregory (12) and Wadley (23) show clearly that poor nutrition of the parent female results in an increase in the proportion of alatae in the offspring.

During the winter period in the heated glasshouse, beans grow spindly and are poor plants compared with those grown in the summer months, due chiefly to the effect of temperature in relation to the winter light conditions. The aphid colonies on these winter plants progress more slowly than in summer, even when summer temperatures are maintained. The developmental period of individuals in winter, when reared under



"summer" temperatures approximated closely to that obtained with summer individuals (effect of temperature), but the aphids individually were not so prolific. Moreover, while with a mean temperature of about 60° F. in winter fewer apterae were obtained, apterae predominated under these temperatures during the summer period if overcrowding was avoided, which suggests the influence of the better nutrition value of the plants in summer. During the winter period with moderate temperatures alatae tended to predominate in the colonies, although with comparatively high temperatures (see p. 121) the influence of the nutrition factor is overcome and apterae predominate. It is interesting to note that the apterae frequently feed below the leaves on these winter plants and not on the growing apex, whereas in summer they invariably feed on the growing tip, unless overcrowded conditions force them to the older parts of the stem and beneath the older leaves. On two or three occasions during the winter period, it was observed that the aphids on bean plants which became sickly owing to root rot developing, left the plant (particularly the apex of the stem) and wandered to the muslin covers. These observations show that the aphids react to the physiological condition of the plant.

From March onwards there is a marked improvement in the growth of the bean plants compared with the earlier period of winter, and as spring advances, if a favourable temperature is maintained, the aphid colonies make better progress and a higher proportion of apterae develop.

(5) *The effect of temperature on occurrence of alatae and apterae.*

It has been shown that during the summer period when the aphids are reared on a favourable food plant (broad beans) and overcrowding avoided, the offspring of apterae tend to be predominantly apterae. Favourable nutrition, correlated with the large area of succulent growth on the plants, is the most important factor favouring the occurrence of apterae during this period, and the moderate fluctuations in the summer temperature do not markedly influence the sequence of alatae and apterae. Alatae, however, predominate irrespective of temperature when overcrowding occurs. During September, under the influence of a falling temperature, shorter hours of daylight, and lack of suitable food-plants (nutrition), the aphid colonies are much smaller and alatae tend to predominate even when overcrowding does not occur and comparatively few apterae may develop. When the aphids were transferred to a warm glasshouse in October, the proportion of apterae increased. In Fig. 3 the results are shown of counts made of apterae and alatae

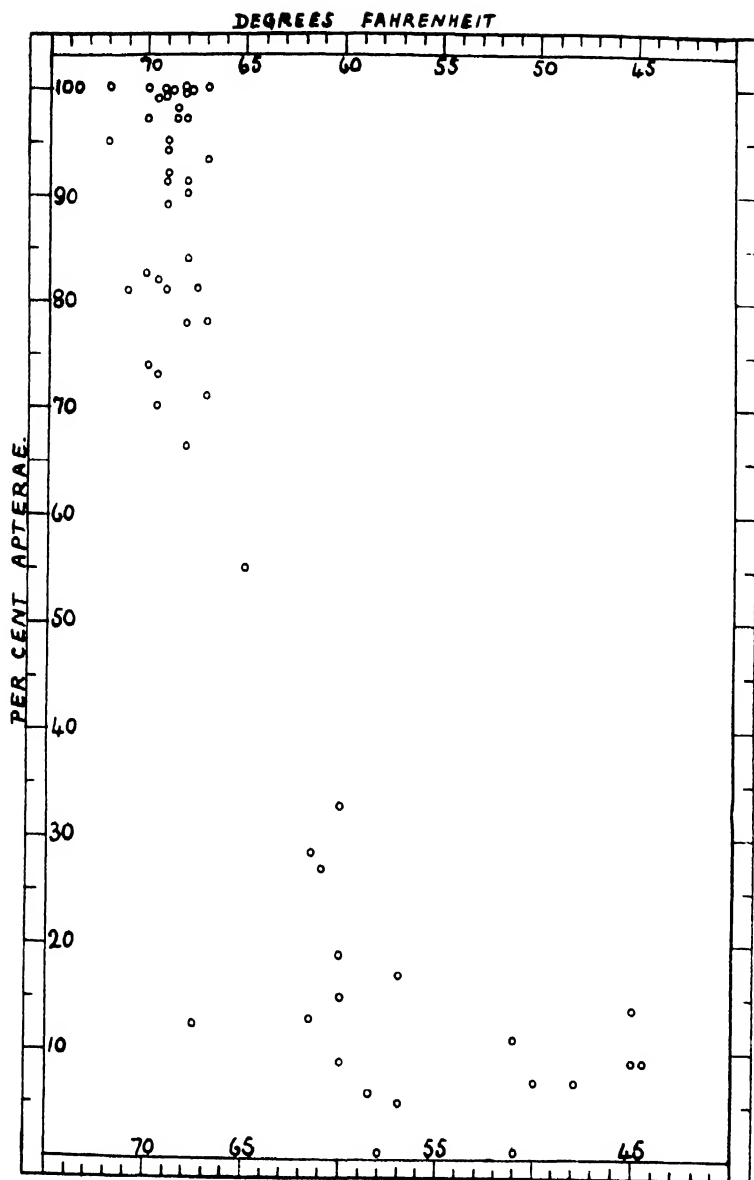


Fig. 3. Showing the influence of temperature on the occurrence of apterae and alatae during the winter period. Counts were made from 56 colonies (offspring of apterae) in lines A, B, C, G, H, Ha during the winter period and the percentage of apterae present in each colony is plotted with reference to the mean temperature of the reproduction period in each case. As far as possible precautions were taken to prevent overcrowding.

## 120 *Parthenogenetic and Sexual Forms in Aphis rumicis*

present in 56 colonies reared under various temperatures during the winter period. The colonies were taken from five of the parthenogenetic lines in different years. They were killed off for counting after a reproduction period of 2 to 3 weeks or longer in the case of the lower temperatures. Precautions were taken to prevent overcrowding and the alatae and apterae were diagnosed as in the experiments referred to in a previous section. With a mean temperature of about 67° F. and over there was a marked increase in the proportion of apterae in the colonies, and below about 57° F. there was a relatively small proportion of apterae. It should be noted that these counts include the colonies which received artificial light, as there was no apparent difference in the proportion of apterae and alatae present compared with the control colonies, as is seen in Table VI. The temperatures maintained in both these sets of experiments were moderately high.

### B. OCCURRENCE OF THE SEXUAL FORMS.

Owing to the large number of colonies which were reared in the various parthenogenetic lines, it is not feasible to present the data in the form of tables, but in Figs. 4 and 5 the occurrence of the sexual forms has been indicated by symbols placed in positions which show the approximate dates when *adult* sexual individuals were recorded in the colonies. Each symbol represents a varying number of individuals present in a colony, so that usually the total number of symbols on any parthenogenetic line shows the number of colonies in which sexual forms were recorded.

It will be seen from Fig. 1 that, with the exception of line H in which the colonies were reared under shorter hours of daylight, *adult* sexual forms occurred with great regularity each year in October, and where the lines were continued parthenogenetically throughout the winter, they appeared from time to time from October until about the end of the following May. It is interesting to note that, although sexual forms developed during the spring and early summer months in overwintered lines, they did not develop in the colonies of lines started from the fundatrix in spring until the following October, although during the spring period both sets of colonies were reared under the same conditions. In the colonies of the overwintered lines there was, however, a progressive increase in the proportion of parthenogenetic individuals present in the colonies as spring advanced, compared with the earlier winter period: alate virginoparae began to appear during March and April, the number of alate sexuparae becoming less, so that about the end of May, the alate forms tended to be entirely virginoparae. The occurrence of the sexuales



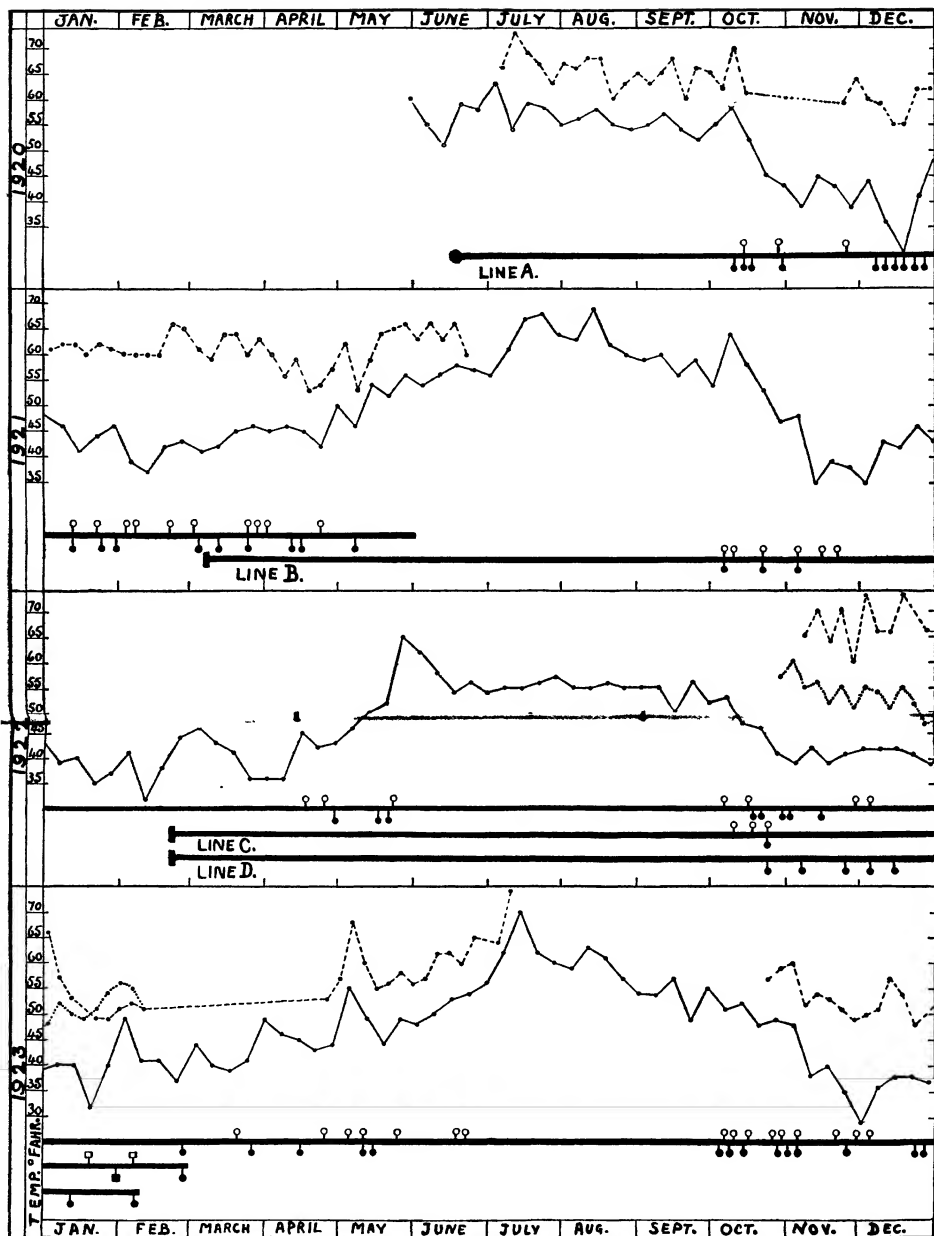


Fig. 4. The chart shows dates on which adult sexual forms of *A. rumicis* were recorded in the colonies of the parthenogenetic lines A-D. The thick horizontal lines show the length of time parthenogenetic reproduction was maintained in each of these parthenogenetic lines. The symbols attached along these lines represent adult males (small open circles above the lines) and adult sexual females (small closed circles below the lines). The places where these symbols are attached indicate the approximate dates these forms were recorded in the colonies. It will be noted that during June to September only parthenogenetic individuals were obtained.

The air temperature at Rothamsted (daily mean of weekly periods) is shown by the whole-line curve; the glasshouse temperature at different periods (daily mean of 5-day periods) is shown by a broken-line curve. During 12. xi. 22 to 12. i. 23 the colonies in lines B and C received artificial light (note absence of sexuales) and temperature as shown in upper curve (broken line). With line C (January 1927) the square symbols indicate occurrence of sexuales in colonies receiving same temperature, but no artificial light. With line D the colonies received only ordinary daylight during this same period, and lower temperatures as shown in middle (dotted line) curve (note occurrence of sexuales).



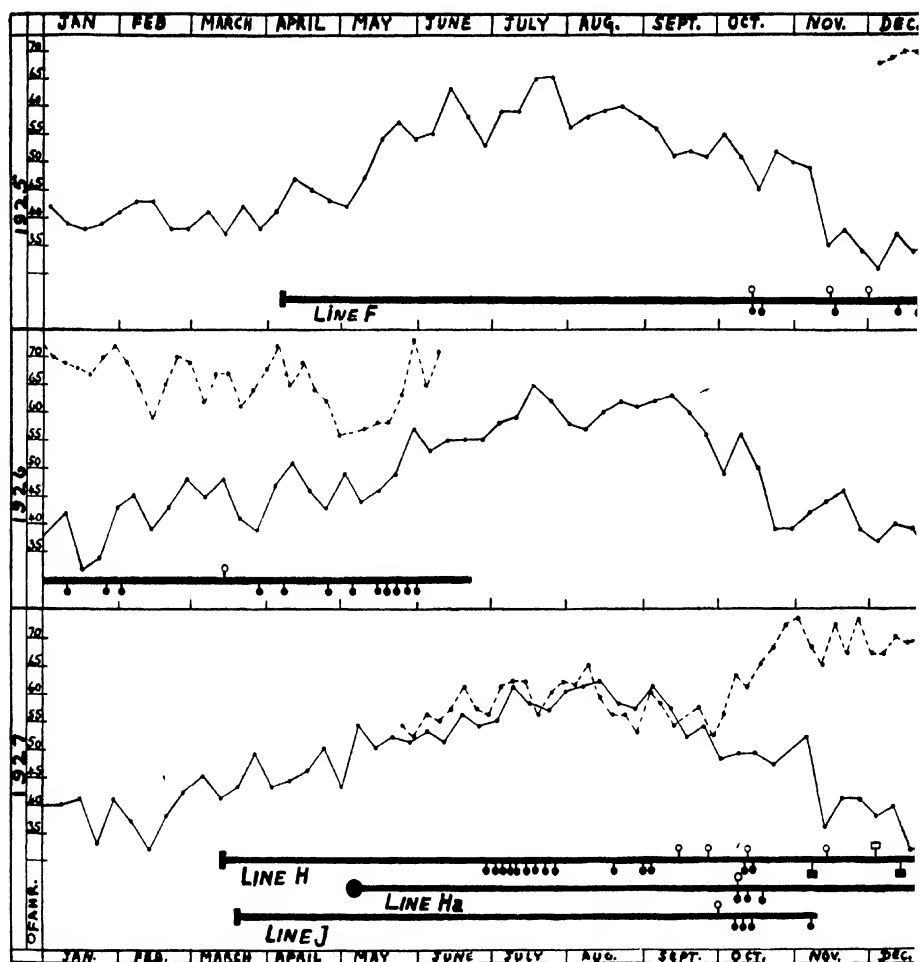


Fig. 5. The plan of this chart which shows the data obtained with the parthenogenetic lines F, H, Ha and I is as described for Fig. 4. The whole-line temperature curve shows Rothamsted air temperature. The broken-line temperature curve in 1927 refers to the open-air insectary (up to 30. ix. 27) and after that date the heated glasshouse.

During the period 28. iii. 27 to 5. ix. 27 the colonies in line H received only 8 hours daylight daily (note early appearance of sexual females). During period 25. x to 23. xii. 27 the colonies in line H and Ha received artificial light in excess of normal daylight (note absence of sexuales): the square symbols indicate occurrence of sexual forms in colonies not receiving artificial light.

From particulars given in Section II of the paper, by referring to Figs. 4 and 5, the reader can see the conditions under which the aphids were reared throughout the year.

Charts IV and V should be compared with Fig. 1.

## 122 *Parthenogenetic and Sexual Forms in Aphis rumicis*

during the spring period indicates a carrying-over effect of the previous winter conditions under which the aphids were reared.

After the sexual forms first began to appear in the various lines, apterae were transferred in successive generations to form new colonies and so continue the parthenogenetic line: the alate offspring were tested out in succeeding generations to see whether they produced sexual females (being sexuparae) or parthenogenetic females (being alate virginoparae). It is necessary in order to make sure that the sexual phase is present or absent, to rear the offspring of these alatae in as many of the successive generations as possible, in order to test whether the latter are sexuparae or virginoparae. In my experiments males were found sometimes to occur somewhat irregularly during the winter period, and one cannot rely upon their non-occurrence as indicating that the sexual phase is absent. Moreover, under the higher temperature conditions during the winter period many alatae died without reproducing, and it was necessary to isolate these forms carefully so as to ensure that they would reproduce. It is due to the precautions taken in this way and to the fact that by rearing a large number of colonies, a representative number of the insects in successive generations were available, that the presence of sexual forms has been demonstrated over such extended periods.

The details regarding the occurrence of the sexuales in the various lines are given below; the dates given are those on which the sexual forms were adult.

1. *Line A.* The sexual phase set in early in October 1920. From October until the following May, 37 colonies were reared and adult sexual forms occurred in most of them on various dates as shown in Fig. 4. After May 1921 no sexual forms occurred.

Males were first recorded on 14. x. 20 and developed in 12 later colonies, the last record being 23. iv. 21. Altogether about 50 males were recorded.

Females were first recorded on 14. x. 20. They appeared in 21 later colonies on various dates, the last date being 7. v. 21. Throughout the winter period, the alatae tested out proved to be only sexuparae, but on 23. iv. 21 both sexuparae and alate virginoparae were present and after this date only virginoparae developed.

2. *Line B.* As this line was carried on through three consecutive winter periods, it will be more convenient to deal with these three periods separately. It will be noted from Fig. 1 that there was a rhythmical



appearance of the sexual forms in October each year and a suppression of the sexual phase from about the end of May onwards, until the following October.

*First winter period 1921-1922.*

The sexual forms appeared early in October 1921 and on various dates until the following May. The temperature in the glasshouse during the greater part of this winter period was comparatively low (about a mean of 50° F.) so that the developmental period of the aphids was long and only 12 small colonies were reared. This explains the few records for sexual forms which were obtained, but the sexual phase was in evidence throughout the period.

Males were first recorded on 6. x. 21 and they occurred in eight subsequent colonies, the last record being 23. v. 22. About 24 individuals were recorded.

Females were first recorded on 6. x. 21 and they developed in five subsequent colonies, the last record being 18. v. 22.

*Second winter period 1922-1923.*

Sexual forms again appeared in October 1922, being present in six colonies during this month and the early part of November. During the period November 8th, 1922 to January 12th, 1923, high temperatures were maintained (see Fig. 4), and the successive colonies (ten in number) received artificial light as explained in Section II. Under these conditions parthenogenetic reproduction was vigorous, apterae predominated and comparatively few alatae developed. Of the latter, some were tested out on 6. xii. 22 and 20. xii. 22 and were found to be virginoparae. With the exception of one male which developed on 29. xi. 22 and one on 6. xii. 22, no sexual forms occurred in these ten colonies.

Five control colonies reared during the same period, under the same temperature, but without artificial light, behaved similarly and no sexual forms developed in them. The results obtained with these control colonies are given in Table VI, together with those of six of the colonies which received artificial light, to show the number of individuals produced under these conditions. These data have been included in Fig. 3.

Five apterae were isolated from one of these control colonies on 6. xii. 22 and placed under lower temperature (middle dotted line in Fig. 4). The majority of their offspring developed into alate sexuparae. It should be noted that even with high temperatures during the winter period—which favours parthenogenetic reproduction—the winter light

Table VI.

*Showing the proportion of apterae produced in colonies of line B during winter period 1922-1923.*

A.L. = artificial light series; N.L. = controls with no artificial light.

Colony no.		Infection with apterae	Reproduction period days	Mean temp. of period ° Fahr.	No. of aphids		Apterae %
A.L.	N.L.				Apterae	Alatae	
51	—	2	21	67	32	0	100
59	—	2	14	68	52	8 (N)	88.6
—	48	2	14	68	20	2 (N)	91
—	54	2	14	68	10	0	100
45	—	5	14	68	40	5	89
60	—	2	14	68	44	0	100
—	49	2	14	68	35	1	97
—	55	2	21	67	25	2	93
52	—	2	21	69	86	8 (N)	91.4
61	—	2	14	67	48	0	100
—	50	2	14	67	20	8 (N)	71.4

conditions tend to exert an influence in favouring the occurrence of the sexual phase as will be discussed later.

After the middle of January 1923 the aphids in line B were reared under lower temperatures. From the middle of January until June 1923, eleven colonies were reared in which males developed on various dates, the first being recorded on 20. iii. 23, the last record being 22. vi. 23. Altogether about 26 males were recorded in this second winter period. Females also developed in five of the eleven colonies referred to above, the first record being 28. ii. 23 and the last 15. v. 23. The alatae tested out after 24. v. 23 proved to be only virginoparae and no sexual forms occurred in the colonies until the following October (third winter period).

#### *Third winter period 1923.*

Males were first recorded on 6. x. 23 and they appeared in seven further colonies on various dates, about 47 individuals being recorded. Females were first recorded on 3. x. 23 and subsequently in six further colonies. The parthenogenetic line was discontinued at the end of December 1923. It will be noted (Fig. 4) that the temperature conditions during this winter period were more favourable for the occurrence of sexual forms than was the case with the somewhat higher temperatures maintained during November and December of the previous winter.

3. *Line C.* Sexual forms appeared in this line in October 1922. During the period November 8th 1922 to January 12th 1923 five

colonies were reared under similar temperatures and artificial light as in line B. The results obtained were similar, sexual forms did not develop, but females occurred later in a continuation colony from this series on 28. ii. 23. Six control colonies were reared during this period under similar conditions but without artificial light, and no sexual forms occurred. However, they appeared earlier in the colonies descended from this control series than in the colonies descended from the series which received artificial light. One male developed on 19. i. 23 and another on 7. ii. 23 and females developed on 31. i. 23. These cases are shown in Fig. 4 by square symbols. Altogether 8 males were recorded in line C. The parthenogenetic line was discontinued at the end of February 1923.

4. *Line D.* In this line adult females were first recorded on 24. x. 22 and they developed in five subsequent colonies, the last record being 8. ii. 23. No males were recorded which is probably due to the fact that only a few colonies were reared and the number of aphids produced was small owing to the lower temperatures (see Fig. 4, middle dotted line). It is interesting to note that, under these lower temperatures, the alatae which developed were only sexuparae and sexual females were produced throughout the period.

5. *Line E.* Males and females were recorded in October and November 1924, and the parthenogenetic line was discontinued at the end of the latter month.

6. *Line F.* Sexual forms appeared about the middle of October 1925 and occurred during November. From 1. xii. 25 onwards throughout the winter period, twenty-six colonies were reared, and sexual females developed at frequent intervals as shown in Fig. 4, the last record being 30. v. 26. The alatae tested out from time to time proved to be sexuparae, but in two colonies on 8. iv. 26 and 29. iv. 26 alate virginoparae were also present and after 17. v. 26 only virginoparae developed, the sexual phase being suppressed. It was particularly noted in this line, during the period December to March, that many alatae died without reproducing, and careful attention was necessary in order to get the alatae to reproduce. In one instance, about 14. xii. 25, one plant was infected four times with a total of 32 alate forms and only 4 individuals were produced, which were sexual females. The temperature conditions evidently favoured parthenogenetic reproduction and the sexual forms obtained were few in number. Even where sexuparae developed, they were induced to reproduce only with difficulty. From December 1st onwards two isolated males were obtained, one on 2. xii. 25 and the other on 15. iii. 26.

It will be noted that the temperature during this winter period was only slightly lower than during the period November 1922 to January 1923, when, in lines B and C, the sexual phase was practically suppressed in the control colonies and completely suppressed in those colonies which received artificial light.

7. *Line G.* Sexual forms appeared in October 1926 and the parthenogenetic line was discontinued early in November.

8. *Line H.* Under the conditions of short hours of daylight in this line (see Section II) adult sexual females were first recorded on 29. vi. 27. From 18. vi. 27 to the beginning of October, eighteen colonies were reared and females occurred in thirteen of these, the last record being 15. x. 27. It was noted that from the beginning of June the offspring of the apterae tended to be alatae, which proved to be sexuparae. Males, on the other hand, were not recorded until 14. ix. 27, that is, about the normal time, others were recorded on 26. ix. 27 and 15. x. 27, in all a total of five individuals.

From October 6th onwards eight colonies were reared under higher temperatures and received artificial light as stated in Section II. Under these conditions parthenogenetic reproduction was vigorous and the aphids which developed were in a large majority apterae. The comparatively few alatae which developed were found to be virginoparae, and no sexual forms were obtained. Nine control colonies were also reared under the same temperatures, but without artificial light. Apterae predominated also in these control colonies and comparatively few alatae developed; those tested out on 15. x and 2. xii. 27 proved, however, to be sexuparae and sexual females developed on 5. xi and 13. xii. 27. One male was recorded on 2. xii. 27. These three instances are shown in Fig. 4 by means of square symbols.

These results support those obtained under similar conditions during November and December 1922 in line B. The evidence from line H shows that favourable temperatures and long hours of daylight favour parthenogenetic reproduction, whereas the reverse conditions favour the sexual phase. Even with favourable high temperatures during the winter period (short hours of daylight), although the temperature favours parthenogenetic reproduction, there is a tendency for the sexual phase to develop, presumably due to the light factor, as is seen in the behaviour of the control colonies in this line.

9. *Line Ha.* This is a control for line H, the aphids being descended from the same fundatrix. Sexual forms did not develop in the colonies

until the middle of October 1927. Females were recorded in three colonies on October 8th, 10th and 17th, and the alatae tested out proved to be only sexuparae. Three males occurred in one colony on 17. x. 27. Colonies from this line were established on *Euonymus*, ova were laid and fundatrices commenced to hatch out on 25. iii. 28.

From the middle of October to the end of December, ten colonies were reared which received artificial light as in line H. The aphids in these colonies were chiefly apterae, very few alatae developed and those tested out on 16. xi, 12. xii and 15. xii. 27 proved to be virginoparae. No sexual forms were obtained.

Eight control colonies were reared during the same period as in line H. Parthenogenetic reproduction was vigorous and no sexual forms were obtained.

10. *Line I.* In this control line sexual forms did not occur until October, females being recorded in four colonies about 10. x. 27 and 14. xi. 27. Three males developed in one colony on 30. ix. 27. The line was discontinued about the middle of November.

## VII. GENERAL CONCLUSIONS AND SUMMARY.

### 1. *Material employed in the experiments.*

Ten related lines of the black bean aphid (*Aphis fabae* Scop. = *A. rumicis* L.), in which continuous parthenogenetic reproduction was maintained for varying periods, have been reared during the past seven years (Fig. 1). The longest period was 2 years 10 months with line B, 50 generations having been passed through. The lines were all of the same strain, nine of them being descended from line B, which was started from one fertilised egg. The remaining lines (except Ha) were similarly started from fertilised eggs.

*Euonymus europaeus* was used as the primary food-plant (winter host) and broad beans as the intermediate (summer) food-plant. During the summer period (April to October) the aphids were reared in a large open glasshouse or in the open-air insectary, and during winter (November to March) in a heated glasshouse with varying temperatures (Figs. 4 and 5). The *Euonymus* plants on which fertilised eggs were laid in autumn were kept outside in the open during the winter period.

## 2. *The normal life cycle of Aphis rumicis L.*

*Aphis rumicis* is a migrating species in England, its usual winter host being *Euonymus europaeus*. Its normal life cycle resembles that of other migrating Aphidini; sexual forms occur about October and the fertilised eggs commence to hatch the following March (Fig. 2).

## 3. *Fundatrigeniae generations.*

The fundatrix is considered in this paper as the first generation. Its offspring are usually apterae but may also include alatae (migrants). The apterae of second generation give rise chiefly to alate migrants and a few apterae. The offspring of the latter forms normally consist of alate migrants so that the fundatrigeniae generations come to an end.

There is an inherent tendency for the offspring of the fundatrigeniae apterae to develop into alate migrants, evidently associated with the evolution of the migrating habit. The proportion of alatae and apterae is, however, influenced by environmental factors, particularly overcrowding and the condition of the food-plant (nutrition)<sup>1</sup>.

## 4. *Alienicolae generations.*

The alate migrants have a strong inherent tendency to migrate from the primary host plant, but if confined on *Euonymus* they reluctantly reproduce on it. Moreover, while their offspring on beans develop into apterae, when produced on *Euonymus* some of them develop into alatae owing to the influence of the nutrition factor. The migrants initiate the alienicolae generations. Under certain environmental conditions the succeeding alienicolae generations may consist only of parthenogenetic individuals, namely apterae and alate virginoparae. Under other conditions sexual forms develop. The latter consist of alate males (offspring of apterae) and apterous females (offspring of alate sexuparae). The proportion of sexual and parthenogenetic individuals which develop depends upon environmental factors: on the one hand we may get the parthenogenetic generations terminated owing to the fact that sexual individuals only are produced: on the other hand, only parthenogenetic individuals may be produced. Conditions may occur under which both types are represented.

<sup>1</sup> In non-migrating species, migrantes in the true sense do not occur and the alatae are to be considered as dispersal forms. Reinhard (19) considers that with *Aphis gossypii* the normal tendency is for the parthenogenetic individuals to be apterous. Baker and Turner (2) showed that with *Aphis pomi* the complete bi-sexual cycle could be completed without the occurrence of alate individuals.

## A. THE OCCURRENCE OF ALATAE AND APTERAE.

Three types of alate parthenogenetic females occur during the complete life cycle, namely (1) migrantes, (2) dispersal forms, (3) sexuparae. These three forms resemble one another morphologically, but they differ in that each type plays a different rôle in relation to the migrating habits of the insect. It is necessary therefore to consider them separately when dealing with the influence of various factors on the occurrence of apterae and alatae.

The migrantes have been dealt with in the previous section as they belong to the fundatrigeniae generations. The dispersal forms and the sexuparae occur in the alienicolae generations.

The alate dispersal forms (alate virginoparae as distinct from the alate sexuparae) are chiefly offspring of apterae. They ensure a wide distribution of the species, but are not essential for the completion of the bi-sexual cycle. Their occurrence depends upon environmental factors, particularly (1) overcrowding, (2) temperature, (3) nutrition. Correlated with their function as "colonisers," their offspring normally develop into apterae which latter are more prolific than the alate forms. Under favourable conditions the offspring of apterae develop into apterae, but with adverse conditions of overcrowding, low temperatures and poor nutrition, alatae may also develop. The proportion of apterae and alatae depends upon the influence of these three factors. Actually during the summer, temperature does not play such an important part owing to the moderate fluctuations about a favourable mean temperature.

The experimental results obtained by Wadley<sup>(23)</sup> and others with other species of aphids show that under the influence of one or more of the factors referred to above, the offspring of apterae (and to some extent alatae) may develop into apterae or alatae. The fate of the individuals in this respect may be determined in the early instars immediately after birth, or during pre-natal development in the parent female. From the embryological studies of Uichanco<sup>(21)</sup> we know that the embryos of the young offspring are well advanced, even in the early instars of the parent female. With the exception of the work of Ackerman<sup>(1)</sup> no detailed investigations have been made on the physiological aspect of the occurrence of apterae and alatae, and it is to be hoped that his interesting observations will be studied further.

The alate sexuparae mark the appearance of the sexual phase. The occurrence of the latter is associated with the seasonal factors of light (length of day) and temperature.

## B. THE OCCURRENCE OF THE SEXUALES.

The rhythmical occurrence of the sexuales in October each year in the various lines (Figs. 4 and 5), indicates that this phenomenon is due to the influence of seasonal factors. Light (length of day) and temperature have been shown to be the important factors. While with normal daylight adult sexuales first occur in October, it was found that in the case of line H, in which the colonies received only 8 hours daylight daily, sexual females first appeared in June and were obtained in subsequent generations throughout the summer.

When the various lines were continued parthenogenetically throughout the winter in a heated glasshouse using normal daylight, sexual forms appeared in the colonies until the following May, provided a moderate temperature (about a mean of 58° F.) was maintained (lines A and D). From March onwards the proportion of parthenogenetic individuals increased and with the exception of two instances in early June, adult sexuales were not obtained during June to September (excluding line H), but they reappeared again in October (line B).

With moderately high temperatures during the winter period (about a mean of 70° F.) sexual forms were obtained only rarely or not at all (lines B, H, indicated by square symbols). There was a high proportion of parthenogenetic individuals under these conditions and apterae predominated.

With slightly lower temperatures during the winter period (line F) a few sexual females were obtained in most generations, but the alate sexuparae did not readily reproduce. Males were rare.

When the colonies were subjected to artificial light in excess of the normal daylight during the winter period, and a mean temperature of about 70° F. maintained, no sexual forms appeared (lines B and C, November 1922 to January 1923 and lines H and Ha, October to December 1927).

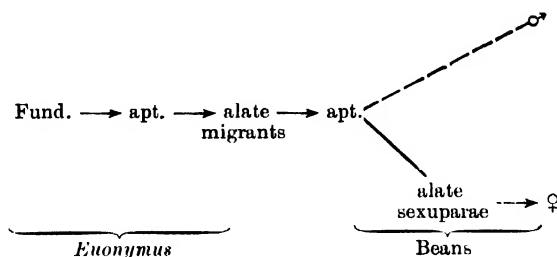
The higher temperatures favour the development of parthenogenetic forms, while the winter light conditions favour the development of the sexual forms. The normal parthenogenetic method of reproduction during the summer, and the suppression of the sexual phase is to be considered as an expression of the influence of these favourable seasonal factors<sup>1</sup>.

<sup>1</sup> The few records of the occurrence of sexuales in tropical and sub-tropical countries does not necessarily indicate a complete absence of these forms in most species. Owing to the favourable environment, parthenogenetic forms may be dominant, and the sexuales comparatively few. Yingling (*Journ. Econ. Entom.* p. 223, 1917) records the sexual females and eggs of *Anoecia corni* in South Texas during December.



From the cytological studies of Morgan, von Baehr, and others it is evident that the mechanism which determines the change from the parthenogenetic to the sexual individual, is associated with the chromosomes. The fate of the offspring of the sexuparae is therefore determined during their pre-natal development. Thus, for instance, although adult sexual forms occurred up to about the end of May, they were probably determined by the conditions obtaining about the end of April. When adult alate sexuparae were isolated in July from colonies in line H and placed under normal daylight conditions, their offspring developed into sexual females. Similarly, during the winter period, when alate sexuparae, reared under temperatures about 58° F., were transferred to higher temperatures, their offspring developed into sexual females.

The fundatrix is also predetermined in the fertilised egg, and Baker and Turner(2) have shown with *Aphis pomi* that the embryo is well advanced by the time the fertilised egg assumes the winter resting condition. With migrating species of aphids like *A. rumicis* it is possible that not only is the fundatrix predetermined but also the occurrence of the alate migrants is inherently established. This would explain why sexual forms did not appear in line H until June. Actually in this instance alate sexuparae developed in the second alienicolae generation as shown below.



Normally, in nature, many alienicolae generations are passed through before the sexuales appear in autumn, and the results referred to above show clearly that the sexual phase develops irrespective of the number of generations passed through. The seasonal factors concerned in affecting the occurrence of the sexual and parthenogenetic phases in the life cycle of *A. rumicis* are shown graphically in Fig. 6, and the dates of the

## 132 *Parthenogenetic and Sexual Forms in Aphis rumicis*

occurrence of sexual forms in the various lines should be referred to this diagram.

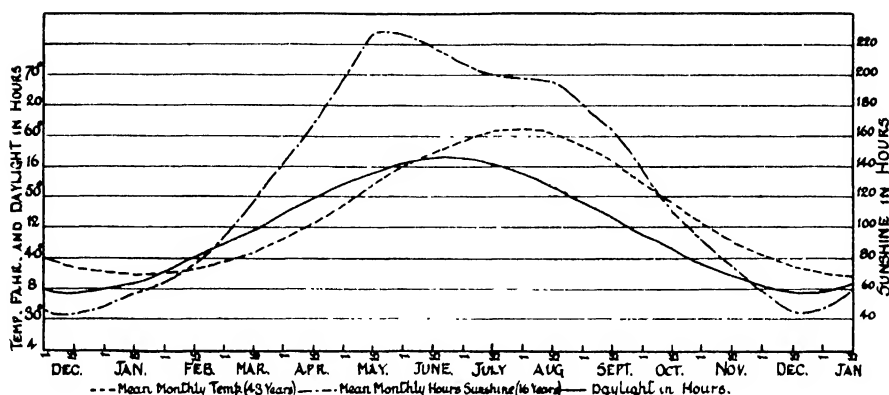


Fig. 6. Diagram illustrating the relation of seasonal factors of temperature and sunshine (Rothamsted Records) and daylight throughout the year. The dates of the occurrence of sexual forms in the various lines of *A. rumicis* should be referred to this diagram.

### SUMMARY.

1. Ten related lines of *Aphis rumicis* L. have been reared in which parthenogenetic individuals were obtained in successive generations extending over varying periods (Fig. 1): the longest period was 2 years 10 months (line B), 50 generations having been passed through.

2. The aphids were reared in an open-air insectary or a large, open glasshouse during April to October (summer period) and in a heated glasshouse with varying temperatures (Figs. 4 and 5) during November to March (winter period): *Euonymus europaeus* was used as the primary host and *Vicia faba* as the intermediate food plant.

3. The life cycle of this species resembles that of the normal type of migrating Aphidini: in S.E. England the fundatrix usually hatches in March and the adult sexuales appear in October.

4. Three types of alate parthenogenetic females occur during the complete cycle, namely migrantes, dispersal forms and sexuparae: these forms resemble each other morphologically but differ in their relation to the migrating habits.

(a) The migrantes may develop as offspring of the fundatrix, but more usually they are offspring of the fundatrigeniae apterae: their occurrence is due to some extent to an inherent established tendency, associated with the migrating habit, but the numbers which may occur in the various fundatrigeniae generations is influenced by overcrowding

and the condition of the food plant (nutrition): they normally produce apterae (alienicolae).

(b) The alate dispersal forms are usually offspring of alienicolae apterae, but may also be produced by the alatae: their occurrence in the various alienicolae generations is affected by overcrowding, nutrition and temperature: they normally produce apterae, but under adverse conditions may also produce alatae.

(c) The alate sexuparae are offspring of alienicolae apterae and their occurrence marks the appearance of the sexual phase; they produce sexual females.

5. With suitable environmental conditions the parthenogenetic alienicolae generations may be maintained experimentally for long periods, and the sexual phase may be induced or suppressed depending on the factors of light (length of day) and temperature. The effect of nutrition (if any) due to the influence of these factors on the plant has not been determined.

6. With the normal seasonal hours of daylight, adult sexual forms appeared regularly in the various lines in October each year. Moreover, they were obtained throughout the winter period until the following May when moderate mean temperatures (about 58° F.) were maintained (lines A and D); with higher mean temperatures (about 70° F.) during this period, they only occurred occasionally (lines B and C, November 1922 to January 1923, and lines H and Ha, November to December 1927); with slightly lower mean temperatures (line F) a few sexual females were obtained in every generation, but males were rare.

7. When the colonies received only 8 hours daylight daily from the fundatrix stage onwards (line H), sexual females developed in June and subsequent months; males did not appear until October.

8. When colonies received artificial light from electric lamps, in addition to the normal hours of daylight, during the winter period (lines B and C, November 1922 to January 1923, and lines H and Ha, November to December 1927) no sexual forms were obtained; the mean temperature was about 70° F. and, as stated in paragraph 6, the sexual forms only occurred in isolated instances in the control plants (received no artificial light) owing to the influence of the high temperature.

## 134 *Parthenogenetic and Sexual Forms in Aphis rumicis*

### REFERENCES.

This list contains only the publications referred to in the present paper. Further references to papers dealing with the influence of external factors on the occurrence of the parthenogenetic and sexual forms will be found in several of the papers given below.

- (1) ACKERMAN, LLOYD (1926). *Journ. Exper. Zoology*, XLIV, 1-60.
- (2) BAKER, A. C. and TURNER, W. F. (1916). *Journ. Agric. Res.* v, 955-93.
- (3) BRITTAIN, W. H. (1922). *Proc. Acadian Entom. Soc.* 1921, 7-29.
- (4) DAVIDSON, J. (1921). *Ann. App. Biol.* VIII, 51-65.
- (5) — (1921). *Sci. Proc. Royal Dublin Soc.* XVI, 304-22.
- (6) — (1921). *Bull. Entom. Res.* XII, 81-9.
- (7) — (1924). *Science*, LIX, No. 1529, p. 634.
- (8) — (1925). *Ann. App. Biol.* XII, 472-507.
- (9) — (1926). *Verh. 3ten Intern. Ent. Kongress, Zurich 1925*, II, 452-7.
- (10) — (1927). *Journ. Linnean Soc. London, Zoology*, XXXVI, 467-77.
- (11) EWING, H. E. (1925). *Amer. Nat.* LIX, No. 663, pp. 311-26.
- (12) GREGORY, L. H. (1917). *Biol. Bull.* XXXIII, 296-303.
- (13) HORSFALL, J. L. (1925). *University of Iowa Studies in Nat. Hist.* XI, No. 2 (New series, No. 87), 1-57.
- (14) MARCOVITCH, S. (1923). *Science*, LVIII, 537-8.
- (15) — (1924). *Journ. Agric. Res.* XXVI, 513-22.
- (16) — (1925). *Ibid.* XXX, 441-9.
- (17) MASON, A. C. (1922-3). *Florida Entomologist*, v, 53, 52; VI, 25-32; VII, 1-7.
- (18) PADDOCK, F. B. (1919). *Texas Agric. Expt. Sta. Bull.* 257, 1-54.
- (19) REINHARD, H. J. (1927). *Ibid.* 353, 5-19.
- (20) SHULL, A. F. (1918). *Amer. Nat.* LII, 507-20.
- (21) UICHANCO, L. B. (1921). *Psyche*, XXVIII, 95-109.
- (22) — (1924). *Philippine Journ. of Science*, XXIV, 143-247.
- (23) WADLEY, F. M. (1923). *Ann. Entom. Soc. America*, XVI, 279-303.

(Received April 24th, 1928.)

## STUDIES ON *OS CINELLA FRIT* LINN.

A REPORT ON CERTAIN OAT VARIETIES IN RELATION TO  
THEIR RESISTANCE TO ATTACK BY THE FRIT FLY IN  
SWEDEN, TOGETHER WITH DATA CONCERNING THE PRO-  
DUCTION OF RESISTANT UTILITY VARIETIES

By NORMAN CUNLIFFE, M.A.

(*Research Officer, School of Rural Economy, University of Oxford.*)

(With 8 Charts.)

### CONTENTS.

	PAGE
I. INTRODUCTION . . . . .	135
II. THE POSITION OF THE FRIT FLY PROBLEM IN 1926 . . . . .	137
III. RESISTANCE TO ATTACK AND ITS IMPLICATIONS . . . . .	139
IV. THE POSITION IN SWEDEN . . . . .	143
V. OUTLINE OF PROJECTED RESEARCH WORK IN SWEDEN . . . . .	145
VI. THE PREVALENCE OF THE FRIT FLY IN SKÅNE, S. SWEDEN . . . . .	146
VII. EXPERIMENTATION IN RELATION TO THE PROBLEM OF RESISTANCE . . . . .	148
(a) Experimental procedure and data . . . . .	148
(b) Discussion of data . . . . .	152
(c) Interpretation of data . . . . .	162
(d) Other observations on varietal resistance . . . . .	163
(1) Large comparative trial . . . . .	164
(2) Data from observation plots . . . . .	165
(3) A Lochovs × Victory crossing and its results . . . . .	166
VIII. UTILISATION OF OBSERVATIONS ON RESISTANCE . . . . .	167
IX. APPENDIX. A SECOND ANALYTICAL METHOD APPLIED TO THE DATA . . . . .	169
REFERENCES . . . . .	169

### I. INTRODUCTION.

METHODS of control most generally advocated are palliative in nature, are primarily adapted for crops cultivated intensively (which permit expenditure on a comparatively high scale) and can only be advocated for these types of crops. The protection of farm crops, produced under extensive systems of cultivation, has been neglected partly because of the adverse economic factor and partly because of the difficulties to be surmounted in exploring any but the direct methods of control. Non-acceptance of the view that insects can be controlled only by direct

methods in practice necessitates a wider outlook on the subject and, consequently, the exploration of the possibilities of indirect methods of control, other than the well-known biological method. As it is, at present, impossible to move the prevalent periods of a pest in time, we must necessarily endeavour either to control the susceptible period of plant growth or to aim at the production of resistant or immune varieties. Preliminary studies of this nature, revolving round the resistance of a plant to infestation, immediately and most strongly emphasise the primary importance of the conception of the plant as the central figure and indicate that, in spite of the urgency of special economic problems, the more distant problems associated with the growth phases of the plants, namely, resistance to infestation, recovery power after injury, etc., must be explored if marked and permanent progress is to be expected.

In this report is described research work which was undertaken by means of a Travelling Research Fellowship in Agricultural Entomology, awarded in 1927 by the Ministry of Agriculture and Fisheries. The investigation was conducted at Sveriges Föreningen (The Swedish Seed Institute), situated in the village of Svalöv, in the southern Swedish province of Skåne. The object of this investigation may with advantage be stated briefly at once. At Svalöv, claims have been made that varieties of oats, which have shown marked powers of resistance to attack by the frit fly, have been and are being established. If these claims could be substantiated then (1) it was urgently necessary to have first-hand knowledge of the factors involved in order to determine whether similar strains or varieties could be utilised in England, and (2) it was equally necessary to study the methods in use at Svalöv in connection with oat breeding, especially in relation to resistance to pests and diseases. On the other hand, if such claims were found to be unsupported by substantial scientific observation, this knowledge in itself would be of considerable importance in England, because we should then be in a position to judge such claims and therefore know to what degree we might expect new varieties to be of value to the English agriculturist.

It is to be regretted that the spring season was the worst experienced in South Sweden since about 1880 and the growing season generally the least favourable for the last twenty years, because these very adverse climatic conditions curtailed the work considerably, and rendered it most improbable that any significant data could be expected to result from plots laid down for recovery trials.

It has not been possible to include in this report data relating to any differences which may be exhibited by the seed of different varieties in

resistance to attack by the frit fly, because the analysis of fifty thousand seeds, for determination of infestation, is a laborious process which will occupy many months.

I have great pleasure in acknowledging herewith the courtesy of Prof. Ehle, the Director of the Institute, and of each member of his staff. They all received me most cordially, provided me with every facility which the Institute could offer and generally extended to me all possible assistance, taking marked interest in this investigation. In particular, I should like to express my very grateful thanks to Dr Åkerman, who is in charge of nearly all the cereal investigations, for his most generous assistance and for placing his extensive knowledge of cereals most freely at my disposal.

## II. THE POSITION OF THE FRIT FLY PROBLEM IN 1926.

The frit fly is an insect which has a wide distribution in corn-growing countries and which is of grave importance because its larvae destroy the growing points of shoots and seeds of the oat plants in spring and summer respectively. Limitations of the activity of the fly by chemical or mechanical means of present applicability is considered to be impracticable because of the low net value of the crop per unit area. Even if a practicable control measure of this nature were to be discovered, because of its palliative nature, efforts to establish effective preventive measures must still be made. Research work both here and abroad in relation to the frit fly is directed towards the discovery of a type of oat plant resistant to the attack of the larva. To attain this end it is necessary to determine (1) whether differences in susceptibility to infestation do exist between plant and plant, either of the same or different races or varieties, or between the growth stages of an individual plant, (2) what the characters are which tend to make a plant resistant or immune to attack, and (3) how such characters are or can be associated with capacity for yielding the maximum amount of grain of desirable quality.

To solve the frit fly problem we must increase our knowledge of plant resistance and combine it with our knowledge of the insect cycle. From these aspects, the outstanding facts discovered recently are as follows:

1. That the maximum prevalence periods of the fly in the field are fairly constant in time from season to season; the three generations of adult flies have appeared in maximum numbers about May 26th, July 15th and August 22nd, every season for the last six years (1,2,3).

2. That the shoot and seed have definite growth stages within which they are most susceptible to attack; the shoot is most susceptible while in the two or three leaf stages; from the four leaf stage onwards susceptibility drops quickly (7, 4); the grain is most susceptible about the time of fertilisation and the highly susceptible period closes before the grain reaches half its normal size (9).

3. That varieties of oat plants do show variation in extents of shoot and grain infestation, when grown under similar conditions (5). Of all the varieties submitted to experiment in England, Goldfinder and Supreme are, as judged by our present system of analysis, the varieties least and most susceptible to infestation, respectively. March sowings, for three or four years past, have shown consistent and significant differences in extent of shoot infestation of the order of 20 per cent. in favour of Goldfinder. Unfortunately, seed infestation is not correlated with shoot infestation. Later sowings in April, designed to throw the plants into the fly period about May 26th, while they were in the early leaf stages, showed that under such conditions there were no significant differences in extent of infestation, i.e. no appreciable differences in resistance. The difference in behaviour observed with the earlier sowings was evidently due to some characters which varied during growth but which at present are unknown.

As far as the spring attack is concerned efforts to solve the problem may be based on the study of either or both of the following characters, namely (1) the greatly increased power of resistance of the plant after it reaches the five-leaf stage, or (2) the power of resistance to infestation exhibited by one variety more than another. The problem would be solved, at least as far as the spring attack is concerned, if the correlation between the maximum fly prevalence period and the plant susceptibility period could be prevented. Total immunity might not always be obtained, because tillering capacity might influence extent of infestation; but we should get a shoot resistance of practical importance, particularly with the grain-producing varieties, by arranging that only the resistant stage of the important primary shoot should be subject to infestation (4). This might be accomplished in either of two ways, namely (1) by allowing the plant to have the longest growing period possible before May 26th and therefore time to produce its resistant stage before the fly becomes prevalent, which may be accomplished by early sowing, or (2) by introducing into the more susceptible heavy yielding types the characters which cause certain varieties to exhibit power of resistance, so that such characters may be effective during the earlier stages of growth.



At present the provision of a sufficiently long growing period is only practicable with spring oats by eliminating sowings after the end of February. Reckoning a period of three weeks for germination and periods of seven days for the production of each susceptible growth stage, these being five in number, the total period required to bring the plant into the five-leaf stage is 8 weeks(4). Adverse climatic conditions, such as abnormally low temperatures during March and April, may easily cause a set-back in plant growth prolonging the duration of one or more of the leaf stages. This necessitates a safety margin, probably of 2 weeks, which brings the total period, from sowing date to date of production of the resistant stage, up to 10 weeks. Thus, calculating from May 26th, it is obvious that the very latest sowing date for spring oats under English conditions should not be later than the middle of March and probably not later than the end of February for there to be any certainty of normal yield. This agrees with practical experience(10). Even then, the possibility of grain infestation has still to be faced, because of the frit fly population carried by the wild grasses, and this of course applies equally to winter sown oats. Provided, however, that the panicles are exerted early, the risk of heavy grain infestation is not great, because the majority of the second swarm of flies appear about the middle of July, at which time the grain should be halfway to maturity, and therefore reaching a resistant stage.

As circumstances often prevent early sowing, we are driven to attempt to solve the problem by the second method, *i.e.* by studying host resistance, hoping thereby to be able eventually to breed a type of plant with resistant powers in its early growth period and capable of giving high yield of quality grain.

### III. RESISTANCE TO ATTACK AND ITS IMPLICATIONS.

It is necessary to understand exactly the meaning attached to the term "resistance." The relation between the factor "resistance to attack" and the measure "yield" also requires consideration, because of the entry of the unproductive shoot into the problem. Efforts at improving a variety aim at producing a type which will bear the maximum weight of best quality seed per unit area. The utility factors, quality of seed and weight of seed per panicle are independent of the number of panicles produced per unit area. The number of panicles produced depends on both the number of plants produced and the number of shoots produced per plant. that is, on inherent characters of the variety causing certain reactions to environmental conditions, spatial conditions being of

paramount importance in this respect. Therefore, in relation to infestation and consequent loss of shoots, the important factor is the number of shoots produced per unit area. A resistant variety must produce the maximum number of shoots, while a utility variety must produce the maximum weight of quality grain per unit area. Thus, from the point of view of resistance, the value of a shoot depends merely on its presence in an uninfested condition, not on its productive capacity at all. Total unproductiveness of a shoot may be regarded, in this connection, as an extreme case of variation in weight of grain produced per panicle. Therefore the quality of resistance must be considered entirely apart from yield. It is, however, of great importance to remember that resistance factors and utility factors may be combined by breeding, and further, that the utility value of the new product must always be judged on the evidence afforded by adequate yield trials in conjunction with observations on seed quality.

We have then to consider what is implied exactly by the term "resistance to attack" and how resistance ought to be measured. It would appear that resistance to attack may be either direct or indirect. In the case of the frit fly, direct resistance means that the shoot can resist larval entry directly or bring about the death of the larva before it can cause material injury. If the possibility of entry is dependent on the presence of such factors then direct resistance may be present or absent only. If, however, it depends on fluctuating characters associated with growth such as cuticular thickness, presence of silica, hairiness of surface, quality of sap, etc., then this type of resistance may appear and vary with age. It has indeed been proved that susceptibility decreases with age of shoot in the case of Abundance oats. The members of a shoot population, apparently uniform, may, in fact, not be so with regard to direct resistance, if the resistance exhibited by the members of the population depends on both these types of factors.

Indirect resistance includes what may be termed the biological factors. A greater capability of producing plants and shoots per unit area, early tillering capacity (thereby providing susceptible shoots for the larvae and allowing the primary shoots to escape), capacity for rapid growth (enabling the shoot to remove its susceptible regions from the path of the larva), and an efficient recovery power are examples of factors which may be included under this heading. Indirect resistance therefore can be expressed only as a comparative measure of inherent capabilities, as it is dependent on environmental conditions and will vary directly with them.

When considering how resistance may be measured, it will be found that a number of variables have to be considered. The number of larvae present may be equally or unequally distributed. If they are unequally distributed in the first place over so small an area as an experimental plot, obviously experimentation will be difficult there. Measurement of distribution can be made by using the same variety in plots over a large area and determining therefrom the distribution of infested shoots. In the opposite case of equal distribution, three other variables present themselves:

A. The larvae present may be

(a) Limited in number over the experimental area, *i.e.* less than the numbers of shoots open to attack.

(b) Unlimited in number over the experimental area, *i.e.* equal to or more than the numbers of shoots open to attack.

B. The shoots presented for attack by any two varieties may be

(c) Equal in numbers per unit area.

(d) Unequal in numbers per unit area.

C. The capability of resistance to attack may vary with variety, from

(e) No direct resistance and no indirect resistance.

(f) Direct resistance only present.

(g) Indirect resistance only present.

(h) Direct and indirect resistance present.

(i) Complete direct resistance, or immunity.

We may now compare the action of two or more varieties under these various possibilities. If resistance is completely absent, infestation will vary from certain equal infestations when the numbers of larvae are limited and the numbers of shoots equal or unequal to complete infestations when the numbers of larvae are unlimited. On the other hand, if resistance is complete, then infestation will be nil. If the larvae are limited in number and the shoots equal in number per unit area, resistance cannot be measured unless the numbers of larvae present and the numbers of shoots open to attack are at least equal, and the same applies to cases in which the shoots present are unequal in number also. If, however, the larvae are unlimited in numbers, resistance can be measured whether the shoots are equal or unequal in numbers..

Direct resistance must be measured by observation of the infestations of uniform populations of shoots, and direct comparisons may then be made. The most uniform populations under natural conditions are

provided by the primary shoots. It is now known that age of shoot is correlated with susceptibility to attack, therefore it is necessary to have some indication of the degree of variation in rates of growth of the primary shoots in the different varieties in order that we may experiment with uniform material when attempting to measure variation in direct resistance. Careful observation has established the fact that, under similar conditions of growth, there are no important differences in the rates of growth of the primary shoots(4). It must be remembered that apparent uniformity in morphological condition does not necessarily imply uniformity in physiological condition. However, some degree of morphological uniformity is as close to the ideal condition as we can get at present, and the use of such apparently uniform material very much simplifies the position. At the same time such a population approximates very closely to that grown under normal field conditions, because in the field it seems probable that the primary shoot is of paramount importance, firstly, because under commercial conditions about 70 to 90 % of the plants of the grain-bearing oat type produce only one shoot and one panicle and, secondly, because the recovery power of the plant, after the growth of its first shoot has been checked, seems to be very variable(4).

Measurements of the resistance of the total number of shoots carried per unit area are necessarily measurements of indirect plus direct resistance, and therefore are comparative measurements applicable to particular conditions only. If the flies are limited in number direct resistance can be demonstrated, but not measured, by observation of differences in infestations, because experiments will give different results according to the numbers of larvae present each year. If the flies are unlimited and the biological factors approximately constant, the observed percentage differences should be of the same order year by year. In the experiments recorded in previous publications(5,6), therefore, indirect plus direct resistance has been measured, because all shoots have been included in the analyses and the regularity of the results allows us to assume that the larvae are always unlimited in number (within the meaning of "unlimited" stated above). It might be advisable, where possible, to use a highly susceptible variety or stage of plant as a control to demonstrate the existence of an "unlimited" number of larvae. The utility value of any variety will depend therefore on its power of resistance, which may be indirect and/or direct, and on its quality. Yield comparisons alone confuse the issue, wrongly giving an impression of progress and thereby delaying the analysis of the problem of resistance to attack.

## IV. THE POSITION IN SWEDEN.

It was naturally expected that the fundamental aspects of the problem of resistance would be in receipt of attention, in view of the claims made by the Swedish investigators, and that they would be in a position to indicate lines of research relating to resistance likely to increase our knowledge of this subject. This expectation was justifiable without intimate knowledge of their conditions of working, but once this knowledge was attained, it became obviously unreasonable to expect assistance along these lines. The Institute derives support largely from the farming element and the associated commercial company, in addition receiving very limited State aid. The primary aim of each investigator is therefore the production and maintenance of increments in yields by the breeding of suitable new varieties, irrespective of the factors concerned in the improvements and, with the limitations of personnel imposed on the Institute by inadequate financial conditions, it is unlikely that they will progress beyond empirical experimentation in the near future. That they have perforce largely to suppress natural inclinations to explore the more distant problems certainly does not imply that the work of this Institute has not markedly influenced the economic aspect of crop production, but merely explains why such limitations are imposed, their presence and necessity not being obvious to the casual visitor whose impressions are created by 40 acres of plot experimentation. A typical example of the Swedish method of judgment of resistance may with advantage be quoted here. During the year 1918 various lines of a cross between Golden Rain and Black Bell II were compared with Black Bell III both for yield and resistance to frit fly attack. The figures quoted in Table I are the results of averaging the data obtained from triplicate plots. The estimation of the extent of the damage was based on visual observation of the number of panicles present, the figures 5-0 representing grading from normal to worthless stands, the departure from the normal being attributed to the frit fly, because the results of its activity had been observed on the plots earlier in the season. It is obvious that the figures quoted only measure the observer's ability to differentiate between variations in stand which may be the effect of indirect resistance only and that the measurements of yield include the effects of variations in resistance, both direct and indirect, presuming the frit fly attacks to have been unequal in the first place.

It is only fair to state that in other cases the extents of the frit fly attacks have been graded as above early in the season and that the

results of such grading have more or less correlated with the yield figures, as might reasonably be expected.

Table I.  
*Swedish variety trial, 1918.*

Variety	Mean yield	Average estimation of damage
Golden Rain $\times$ Bell II ( $F_5$ )	0.520	3.7
Another line of same	0.542	4.2
"    "    "	0.590	3.8
"    "    "	0.395	3.0
"    "    "	0.230	1.8
Golden Rain	0.190	1.7
Bell III	0.450	4.0
Golden Rain $\times$ Bell II ( $F_5$ )	0.220	2.3
Another line of same	0.205	2.7
"    "    "	0.420	3.3
"    "    "	0.425	3.7
"    "    "	0.360	2.5
"    "    "	0.160	2.2
"    "    "	0.525	4.3
"    "    "	0.600	3.5
Golden Rain	0.160	1.3
Bell III	0.510	3.8
Golden Rain $\times$ Bell II ( $F_5$ )	0.430	3.2
Another line of same	0.375	2.5
"    "    "	0.340	2.0
"    "    "	0.210	1.8
"    "    "	0.760	4.3

From long observation of oat varieties produced by themselves or obtained from other parts of the world, they rightly deduce that constantly high yielding varieties are resistant under their conditions, and it may well be that the inferences drawn from such data would prove on investigation to be valid under other environmental conditions, but it is essential to realise that it is not justifiable to draw general conclusions from such data, because the important factor may be indirect resistance, which itself may vary under varying conditions of fly population and plant growth, such as will occur in other seasons or in other countries. Direct resistance must be proved to be present or the total resistance of each of such varieties must be determined in any new environment, before new varieties can be accepted with confidence for utilisation under other environmental conditions.

The problem of resistance having been studied at the Institute at the best very indirectly, considerable adjustment of the projected lines of

investigation was necessary. Instead of being in a position to study the more distant problems, it was necessary to establish the existence of variation in power of resistance between varieties, under Swedish conditions of growth and fly population. The first step was to make acquaintance with the biology of *O. frit* as known in Sweden. At Svalöv the results of the activity of the fly were of course well known, but accurate and detailed biological data were lacking. The Entomological Institute (Centralanstaltens Entom. Avdelning) at Stockholm was visited at the end of April, as it was understood that Mr Lindblad was engaged in research on this problem, under the direction of Prof. Tullgren. Here it was found that sweepings had been made in the field during the year 1926, but that the material obtained was still awaiting examination; also that numerous parasites had been bred from the immature stages, these parasites being undetermined. It would have been very helpful if their researches into the biology of this pest had been prosecuted further, but other interests claimed their attention.

#### V. OUTLINE OF PROJECTED RESEARCH WORK IN SWEDEN.

The following scheme of work was outlined after the problem had been discussed with Dr Åkerman and the possible scope under the particular conditions determined:

(a) Data as to fly prevalence were to be collected by sweeping in the field.

(b) Early and late sowings of oat varieties were to be made, utilising available stocks of markedly different varieties, for the purpose of determining variations in power of resistance of primary shoots, total shoots and seed, to frit fly attack.

(c) The later sown plots were to be laid down with the intention of obtaining some preliminary information as to recovery power as determined by yield.

(d) Large comparative trials were to be laid down for confirmation of the results obtained from (c).

(e) Records of rates of growth were to be made.

(f) Crosses were to be made between varieties showing marked resistance to infestation and varieties having desirable yield qualities.

(g) Observations were to be made on other material laid down for the general purposes of the Institute work, where it was considered they might provide supplementary data.

## VI. THE PREVALENCE OF THE FRIT FLY IN SKÅNE, S. SWEDEN.

Sweeping was conducted with a conical net, 12 in. in diameter, having a terminal tubular tin to facilitate the killing and transference of material collected. Fifty semicircular sweeps, as far as possible of equal value,

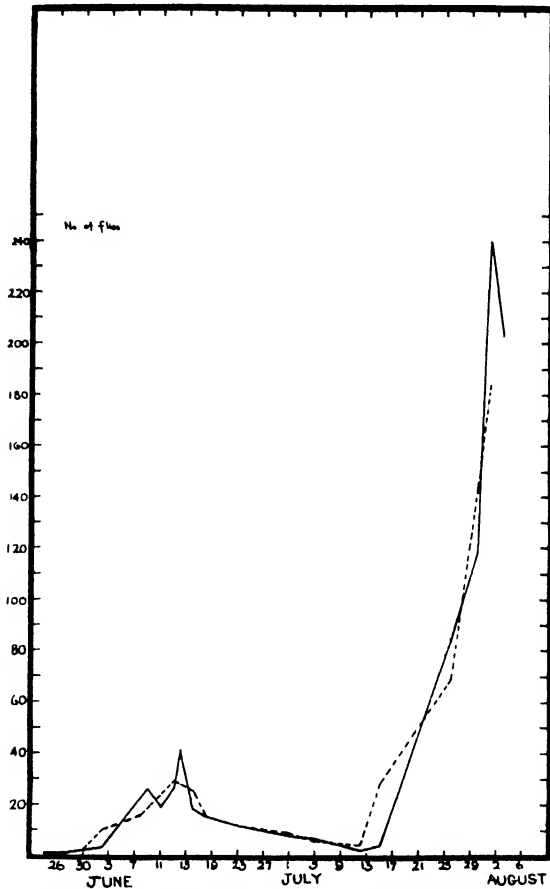


Chart I. Prevalence curve; unbroken line, from actual observations; broken line, smoothed curve.

were made over the tops of the oat plants, the same line in the field being kept on each occasion. As far as weather conditions permitted, collections were made on alternate days and the numbers of flies recorded. These data are set out in Chart I in the form of a smoothed curve, the adopted mean for any one date being the mean of the observation



recorded for that date and the two observations immediately adjacent. Smoothed curves for rainfall and maximum and minimum shade temperatures are shown on Chart II for record.

Previous to May 23rd no flies were swept from any cereal or grass crops, either in the neighbourhood of Svalöv or elsewhere. The latter part

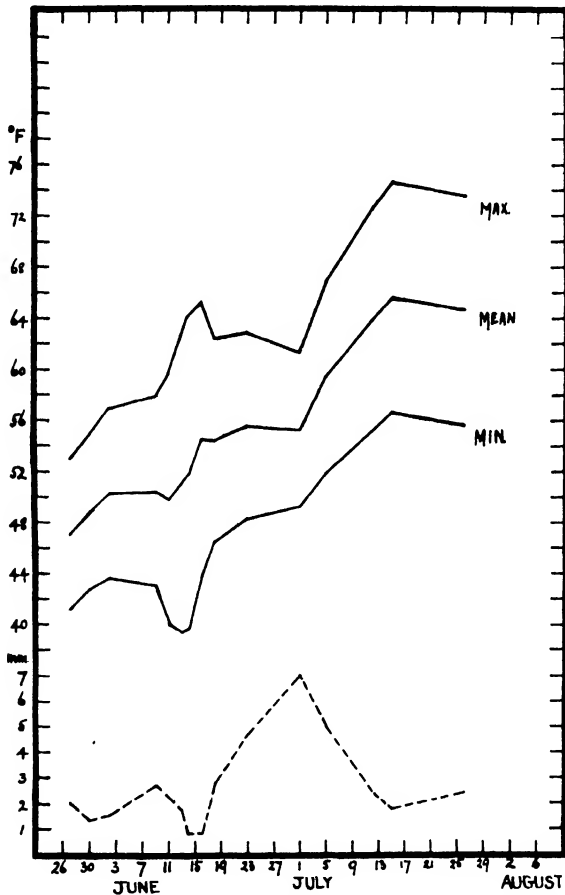


Chart II. Smoothed curves, representing, from below upward, rainfall in mm., minimum, mean and maximum shade temperatures.

of May was decidedly wet, but warm dry intervals were sufficiently numerous to show that emergence did not occur prior to May 23rd at Svalöv. The month of June was very favourable for sweeping, until about the 20th, when continuous rains and high winds prevented observations by this method. The flies were however observed at work on the

experimental plots during this wet period. Very few flies were present in the field during the first half of July and after the middle of the month frequent thunder showers prevented sweeping operations until the 26th, about which time the second swarm of the year began to emerge. The primary object of this operation, namely, the determination of the prevalence of the first swarm of the year, had been attained, but it is to be regretted that it was not possible to complete the observations at this later period. It would appear, from these data, that the duration of the period between the two maximum prevalence periods would have been about 50 days, as it is in England. The mean shade temperature over this period was 60.0° F.

## VII. EXPERIMENTATION IN RELATION TO THE PROBLEM OF RESISTANCE.

### (a) EXPERIMENTAL PROCEDURE AND DATA.

The vagueness of the data relating to the biology of the frit fly made it difficult to prearrange the sowing periods, so in order that it should be certain that the susceptible stages of plant growth should be produced while the flies were in active oviposition, the first sowing was made at hazard on April 25th to 28th, and the second when the first fly was caught in the field, namely, on May 23rd.

Thirty-two varieties of oats, markedly different from one another in various characters such as yielding capacity, colour, tillering capacity and country of origin, etc., were suggested for experiment by Dr Åkerman, who has been making annual visual examinations of scores of different types for many years; these varieties he selected as likely, from such experience, to be productive of result, direct or comparative. At the same time, limitation of experimentation was imposed by two conditions, namely (1) that the supply of labour, both for field work and analysis, was strictly limited, and (2) that it was considered desirable to utilise only such varieties as would be likely to produce, on crossing, types of value to the English agriculturist. The following thirty-two varieties were used in these sowings, namely: King (01171 b); Star (01182); Victory (0355); Golden Rain (0386); Golden Rain II (01221 c); Ligowo (0353), originally from Russia; a cross between Lochows Yellow and Victory (01272); Lochows Yellow, a German yellow oat; Lüneburger Kley, common north of Hamburg; Leutewitzer, an old German yellow oat; Weibull's Echo and Weibull's Early, both lines from a cross between Golden Rain and Leutewitzer; White Yeoman (01164 c); selected Dala (0924), a country variety from middle Sweden; Gophers, from U.S.A.;

White or Kytö, from Tammisto, in Finland; Spet, a country variety from Småland, Sweden; Hede, from Denmark; Summer, from Gotland, in the Baltic; Black Bell II (0408); Engelbrekt (01150 e); Great Mogul (0450); Weibull's Argus; Roslags, a country variety from an island east of Stockholm; Plume or Black Tartar (0210); Black Supreme; Orion II (01104); Mesdag, a Finnish country oat, selected in Holland; Sandy; Tam Finlay; Kent Berlie; and finally *Avena fatua*<sup>1</sup>.

The seed was sown by means of the sowing board in the normal manner, at a spacing of  $12.5 \times 5$  cm. and a depth of 5 cm. on plots carrying four rows, each of 30 seeds, for each variety. The order of sowing was that indicated in the above list of varieties, except that additional plots of Victory oats were inserted to act as controls, the location of this variety being in the plots numbering 3, 13 and 26 respectively. The earlier sowing (April 25th) was replicated twice and the later sowing (May 23rd) five times, the unequal division being due to the limited supply of labour available, to the fact that it was obviously more advantageous to concentrate on the later sown plots, which were certain to be attacked, and finally to the fact that it was desired to obtain from these plots some information as to the recovery power of the different varieties, for which purpose it was essential to have at least six observations of yield for analytical purposes. By May 12th the majority of the first sown plots were showing through, Black Supreme alone failing to appear by this date<sup>2</sup>. The latter part of May, as may be seen by reference to Chart II, was very cold and wet and these conditions were reflected in the poor germination of the seed in this first series. From 120 seeds, the minimum, mean and maximum numbers of plants obtained per plot were 39, 79 and 110 respectively. The plants from the later sowing commenced to break through on June 1st, being produced much more evenly than those of the first sowing, owing to the better climatic conditions prevailing. In this case, from 120 seeds, the minimum, mean and maximum numbers of plants obtained per plot were 32, 102 and 118 respectively. The rate of growth of the primary shoots was determined by observing the date of appearance of the leaves in order, in particular of certain varieties considered by Dr Åkerman to be early in habit. Typical observations made on these particular varieties are shown in Table II.

Late germinations were responsible for the deviations of Golden Rain, Summer, Orion, and Mesdag on May 25th. The early start of such varieties as Lochows Yellow, Gophers, Summer and Mesdag oats was

<sup>1</sup> A number following the name of a variety indicates the Svalöv identification number.

<sup>2</sup> This seed was old and therefore many were not viable.

Table II.

*Showing percentages of plants in different leaf stages at different dates.*

Variety	Percentage of plants in second-leaf stage			Percentage of plants in third leaf	Percentage of plants in fourth leaf
	May 18	May 21	May 25	May 30	June 4
Victory	0	59	100	79	87
Golden Rain	0	63	94	61	84
Golden Rain II	16	96	100	79	92
Lochows Yellow × Victory	21	71	100	93	93
Lochows Yellow	55	91	100	96	88
Weibull's Early	21	93	100	97	83
Gophers	48	87	100	91	78
Spet	33	89	100	89	90
Summer	57	70	96	96	96
Engelbrekt	14	71	100	91	75
Orion	14	94	94	88	82
Mesdag	87	87	85	79	87
Tam Finlay	14	76	100	95	97
<i>Avena fatua</i>	0	20	100	60	39

not maintained, no differences in rates of growth of practical importance being exhibited by the varieties; *Avena fatua* was exceptional, but even in this case, the lag was comparatively slight.

With the later sowings, the plants were in the second-leaf stage by June 7th, in the third-leaf stage by June 14th and in the fourth-leaf stage by June 18th, the rate of leaf production being very even, varieties such as Mesdag being no more advanced than any others. Other observers from members of the staff confirmed the absence of material variation in the rate of growth of the primary shoot and, as all these observations confirmed detailed observations of the same type made in England during the years 1925 and 1926 (4) it was considered unnecessary to amplify them.

The primary shoots of the plants in the earlier sown plots passed from the first- to the fourth-leaf stage during the period May 18th to June 4th. Reference to Chart I will show that these shoots were hardly exposed to the attack of the fly before they reached the stage of comparative immunity. Their tillers, however, were continually exposed to attack. In the case of the later sown plants, the primary shoots were in their most susceptible stage, namely, the early three-leaf stage, on June 14th, at which period the prevalence of the flies was at its maximum.

On June 11th the primary shoots of the plants of the first sowing were marked with red wool for later identification, as they were then in the fifth- and early sixth-leaf stages. The later sown plants were only in the

late two-leaf on this date and as it was considered that their identification would not be difficult when it came to analysis they were left unmarked.

The analysis of the shoots for estimation of extent of attack was commenced on July 1st, that is, about 17 days after the period of maximum prevalence of the earliest swarm of flies, by which time the results of oviposition should theoretically have been apparent visually, but to avoid possibility of error in this direction each shoot was split and when necessary examined under the binocular microscope. Samples, consisting of the products of one drill, were drawn from each plot of each variety and

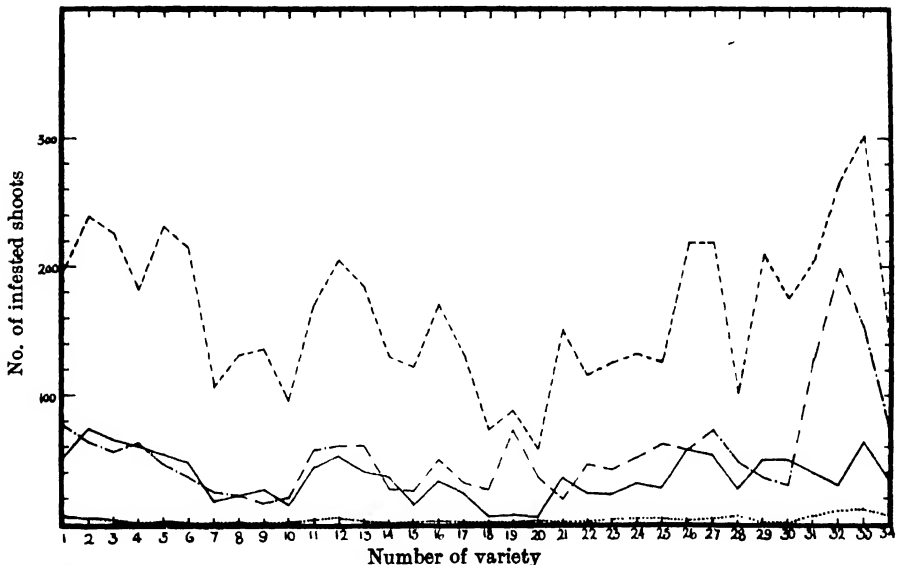


Chart III. Order of infestation shown by each variety; first sowing, three series and second sowing, six series; dotted line=first sowing, primary shoots; broken dotted line=first sowing, total shoots; unbroken line, second sowing, primary shoots; broken line, second sowing, total shoots.

examined in regular sequence, in order to overcome the error which would otherwise have been introduced by late hatching in conjunction with delayed examination. All the varieties composing one series were sampled at the same time.

The necessity of ordered sampling and analysis becomes obvious when it is considered that this analysis alone involved the individual examination of 36,800 shoots. The minimum, mean and maximum numbers of shoots examined in each series were as follows: *first sowing*, primary shoots, 19, 55 and 78; total shoots 102, 315 and 683: *second sowing*, primary shoots,

79, 152 and 178, total shoots, 242, 561 and 813, respectively. It should be noted that the plot replications numbered two for the first sowing, but five for the second sowing. Records were kept of the numbers of unattacked and attacked primary shoots and also the numbers of unattacked and attacked visible or total shoots per plant. The order of the infestation for each series is shown graphically in Chart III, in the form of curves, the actual numbers of infested shoots observed for each variety being indicated. The order of the plots<sup>1</sup> in the field may be indicated as follows:

II 21	II 22	V 25	V 26	VIII 29	VIII 30
↑	↑	↑	↓	↑	↓
I 1	IV 5	IV 6	VII 9	VII 10	IX 36

In Table III are shown the percentage infestations of the shoots for the varieties of oats under observation, in the order of sowing, together with the standard errors of same. The percentage infestation has been derived from the summation of the data obtained from all the plots of the same variety grown under the same conditions, on the assumption that the fly population was uniformly distributed and therefore that each plot could be treated, legitimately, as a sample from a large area. The size of the shoot population has been brought into the determination of the standard error by the utilisation of the formula  $e = \sqrt{P \frac{(100 - P)}{n}}$

where  $P$  = the percentage infestation and  $n$  = the size of the shoot population. A second analytical method is indicated in Section IX.

#### (b) DISCUSSION OF DATA.

The plots of Victory oats numbered twenty-seven and as they were distributed over the whole area, it is possible to determine approximately the type of distribution of the attack and, therefore, of the fly, over the area. The actual numbers of infested primary and total shoots are shown in Chart IV for each plot together with smoothed curves indicating the results of averaging the members of the series in groups of five.

The actual numbers of infested shoots were, as usual, very variable in all the series. How far this was due to inadequate sampling, the irregularity of oviposition or to variation in plant characters, it is impossible to determine in this case, but the necessity for statistical examination of any results, deduced from data of this type, is very obvious. The

<sup>1</sup> Actually there were thirty-six plots in each series, because the field plots numbering 31 and 32 were put down to very early maturing barleys, namely, Six-row barley from Norrbotten (N. Baltic region) and Golden Barley, for the information of Dr Åkerman. Neither of these barleys suffered infestation of practical importance, only a few odd stems containing larvae.

Table III.

*Percentage infestation with standard error.*

Variety in order of sowing	First sowing		Second sowing	
	Primary shoots	Total shoots	Primary shoots	Total shoots
1. King	12.2 $\pm$ 5.1	30.2 $\pm$ 2.9	31.1 $\pm$ 3.6	33.8 $\pm$ 2.0
2. Star	7.3 $\pm$ 3.5	21.2 $\pm$ 2.3	43.2 $\pm$ 3.8	41.5 $\pm$ 2.0
3. Victory	5.6 $\pm$ 3.1	17.9 $\pm$ 2.2	38.2 $\pm$ 3.7	36.2 $\pm$ 1.9
4. Golden Rain	0	22.2 $\pm$ 2.5	34.4 $\pm$ 3.6	32.7 $\pm$ 2.0
5. Golden Rain II	2.8 $\pm$ 1.9	13.4 $\pm$ 1.8	32.6 $\pm$ 3.6	36.6 $\pm$ 1.9
6. Ligowo	1.8 $\pm$ 1.8	14.4 $\pm$ 2.2	28.1 $\pm$ 3.4	36.3 $\pm$ 2.0
7. Lochows $\times$ Victory	0	6.3 $\pm$ 1.2	11.8 $\pm$ 2.5	19.3 $\pm$ 1.7
8. Lochows	1.5 $\pm$ 1.5	6.6 $\pm$ 1.4	14.0 $\pm$ 2.8	24.7 $\pm$ 1.9
9. Lüneburger Kley	0	5.0 $\pm$ 1.2	17.2 $\pm$ 3.0	24.4 $\pm$ 1.8
10. Leutewitzer	2.7 $\pm$ 2.7	8.9 $\pm$ 1.9	12.5 $\pm$ 3.0	20.8 $\pm$ 1.9
11. Echo	4.7 $\pm$ 2.6	16.5 $\pm$ 2.0	31.2 $\pm$ 3.8	35.4 $\pm$ 2.2
12. Early	6.7 $\pm$ 2.9	15.8 $\pm$ 1.9	30.6 $\pm$ 3.5	33.3 $\pm$ 1.9
13. Victory	3.3 $\pm$ 2.3	18.6 $\pm$ 2.1	23.6 $\pm$ 3.2	30.0 $\pm$ 1.8
14. White Yeoman	0	7.1 $\pm$ 1.7	21.2 $\pm$ 3.1	24.4 $\pm$ 1.9
15. Dala	2.4 $\pm$ 2.4	6.7 $\pm$ 1.6	11.2 $\pm$ 2.7	23.7 $\pm$ 1.9
16. Gophers	3.2 $\pm$ 2.2	13.4 $\pm$ 1.7	19.9 $\pm$ 3.0	25.5 $\pm$ 1.7
17. Kytö	1.5 $\pm$ 1.5	10.1 $\pm$ 1.7	14.3 $\pm$ 2.7	25.2 $\pm$ 1.9
18. Spet	1.8 $\pm$ 1.8	7.8 $\pm$ 1.5	5.0 $\pm$ 2.0	13.4 $\pm$ 1.5
19. Hede	1.4 $\pm$ 1.4	13.1 $\pm$ 1.4	5.2 $\pm$ 1.9	12.3 $\pm$ 1.2
20. Summer	4.0 $\pm$ 2.8	11.8 $\pm$ 1.8	3.8 $\pm$ 1.7	11.2 $\pm$ 1.4
21. Black Bell II	5.3 $\pm$ 5.1	18.6 $\pm$ 3.8	22.3 $\pm$ 3.3	30.2 $\pm$ 2.0
22. Engelbrekt	1.5 $\pm$ 1.5	14.0 $\pm$ 1.9	14.1 $\pm$ 2.7	20.8 $\pm$ 1.7
23. Great Mogul	8.3 $\pm$ 4.6	21.2 $\pm$ 2.9	13.8 $\pm$ 2.7	22.2 $\pm$ 1.7
24. Argus	8.3 $\pm$ 4.0	23.2 $\pm$ 2.8	21.2 $\pm$ 3.4	32.4 $\pm$ 2.3
25. Roslags	5.2 $\pm$ 2.5	14.4 $\pm$ 1.7	15.7 $\pm$ 2.7	21.6 $\pm$ 1.7
26. Victory	3.4 $\pm$ 2.4	19.0 $\pm$ 2.2	33.0 $\pm$ 3.5	35.2 $\pm$ 1.9
27. Black Tartar	5.8 $\pm$ 2.8	21.4 $\pm$ 2.2	38.3 $\pm$ 4.1	40.3 $\pm$ 2.1
28. Black Supreme	20.0 $\pm$ 7.3	27.1 $\pm$ 3.3	33.8 $\pm$ 5.3	41.8 $\pm$ 3.2
29. Orion	2.1 $\pm$ 2.1	17.1 $\pm$ 2.6	29.4 $\pm$ 3.5	34.8 $\pm$ 1.9
30. Meedag	0	13.9 $\pm$ 2.3	36.8 $\pm$ 4.1	40.4 $\pm$ 2.4
31. Sandy	12.2 $\pm$ 4.7	33.8 $\pm$ 2.4	33.9 $\pm$ 6.2	34.2 $\pm$ 1.9
32. Tam Finlay	15.2 $\pm$ 4.4	29.1 $\pm$ 1.7	21.2 $\pm$ 4.8	32.6 $\pm$ 1.6
33. Kent Berlie	20.3 $\pm$ 5.5	36.6 $\pm$ 2.4	41.9 $\pm$ 5.7	42.5 $\pm$ 1.8
34. Avena <i>faiua</i>	21.8 $\pm$ 7.3	29.9 $\pm$ 2.9	44.3 $\pm$ 5.6	37.0 $\pm$ 2.3
35. Victory (plots 3, 13 and 26)	4.1 $\pm$ 1.5	18.5 $\pm$ 1.3	31.5 $\pm$ 2.0	33.7 $\pm$ 1.1

smoothed curves rise quickly in the cases of the total shoots and very slightly in the case of the primary shoots of the second sowing. This may mean that the attack was more concentrated on certain parts of the area or that the measurable attack increased with delayed examination. Other varieties occupying intermediate positions, such as Kent Berlie and

Golden Rain II, suffered equally as severely as Victory, tending to show that the attack was not particularly concentrated. The comparative regularity of the mean curve for the primary shoots of the later sown plants, which reached the fourth-leaf stage about the middle of June and therefore would be practically immune by the middle of July, proves that the indicated rise in infestation was due to delayed sampling. The analyses were necessarily made single-handed and occupied a period of about three weeks. It is of importance to know to what degree the flies are limited in numbers in the field because, when studying differences in resistance,

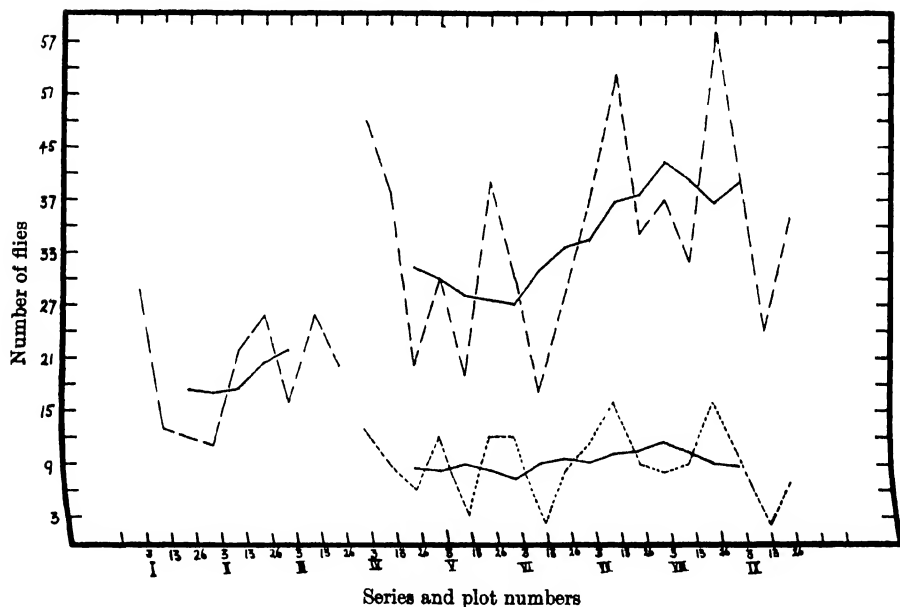


Chart IV. Distribution of infestation, as shown by plots of Victory oats. Dotted line, second sowing, primary shoots; broken lines, first and second sowings, total shoots; unbroken lines, mean curves.

it is essential to know that all susceptible shoots have been exposed to attack. If the number of shoots ( $T$ ) is constant, then the percentage infestation  $P = \frac{I}{T} \times 100$  will increase directly with the actual infestation ( $I$ ). If the number of flies in the field is limited, then  $I$  will become constant for the highly susceptible varieties when their susceptible shoots number more than the larval supply and  $P$  will also become constant, unless  $T$  is also variable. In plot experiments of this type  $T$  is likely to vary but little from plot to plot for most of the varietal types used for



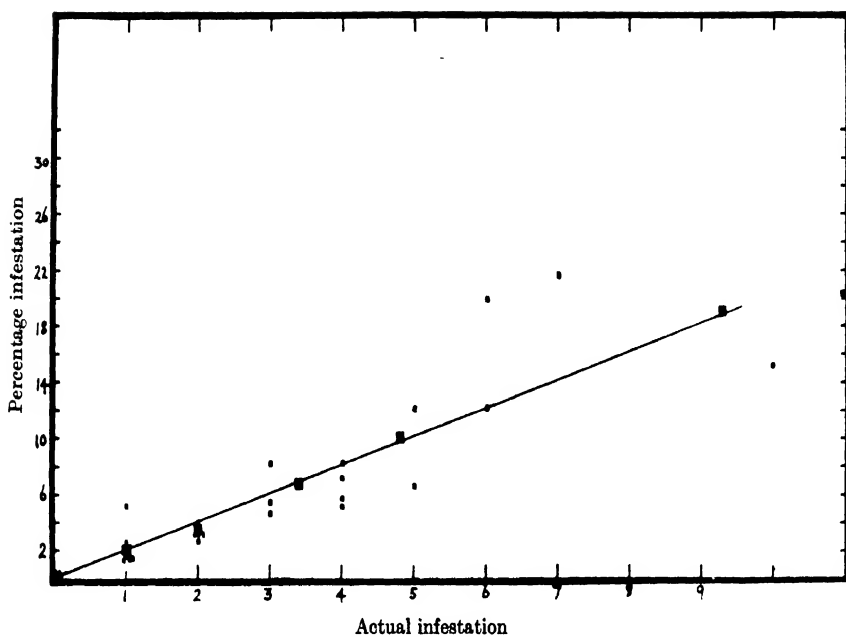


Chart V. First sowing, primary shoots.

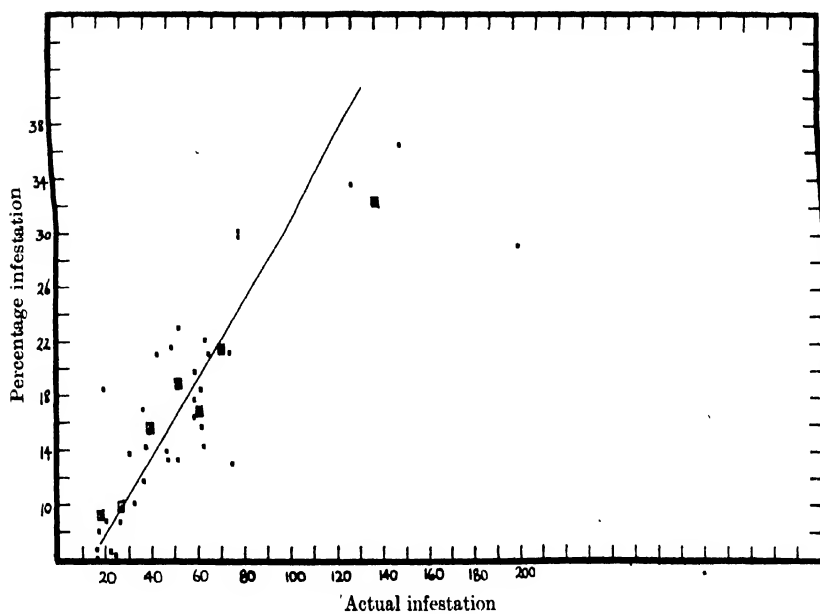


Chart VI. First sowing, total shoots.

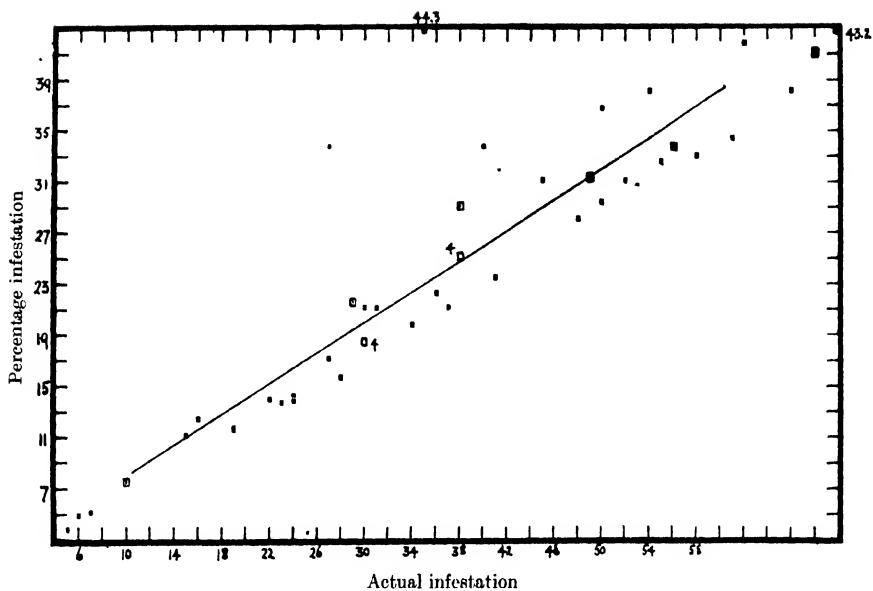


Chart VII. Second sowing, primary shoots.

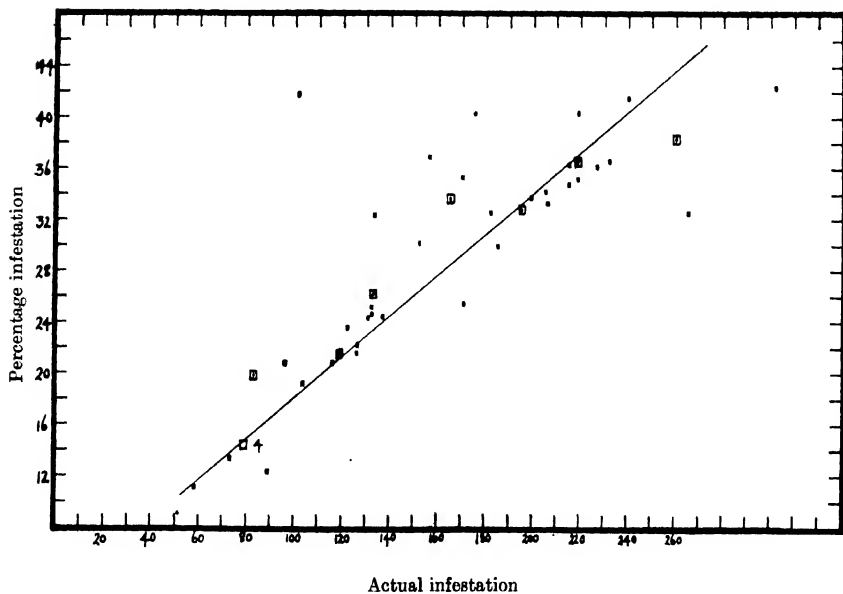


Chart VIII. Second sowing, total shoots.

experimentation, and any indication of the constancy of  $I$  will probably imply a limitation of the fly population. If  $T$  varies, then the curve of  $P$  will tend to depart from the straight line and become parallel to one of the axes. In Charts V to VIII the percentage infestations have been plotted against the actual infestations for each series and the mean curves plotted by taking the means of the observations in sets of five. It will be noted that the mean curves are in the form of straight lines, showing that for the majority of the varieties sufficient numbers of larvae to attack the susceptible shoots were present. If the larvae were limited in numbers, then the mean curve should tend to become parallel to the percentage axes,  $I$  then being a constant and  $T$  variable. None of the mean curves show any very obvious departure from the straight line and therefore it would seem justifiable to assume that the larvae were unlimited, *i.e.* present in sufficient numbers to attack all the susceptible shoots present in the majority of cases. Isolated records well below the mean curve signify that for that particular case the larvae were limited in numbers and therefore that the true susceptibility was not being measured. A case in point is shown on Chart VI, where Tam Finlay, with an actual infestation of 199, shows a percentage infestation of only 29.1. What actually occurs is that tillering is rapid when the prevalence of the fly is on the wane. A record well above the mean curve implies that the stand of shoots was much below the normal and probably also that their growth was deficient, therefore their susceptibility high. These facts may be expressed numerically by the coefficient of correlation between percentage infestation and actual infestation, as shown in Table IV. These indices also show that resistance may be measured either by the percentage or the actual infestation, when the larvae are unlimited in numbers, and suggest that the importance of tillering capacity still requires detailed study from this point of view (section C). The low indices obtained within the comparative series are due to the unequal occurrence of tillering without equivalent exposure to infestation.

The distribution of the plots of Victory oats was arranged to determine the variation over the area in the distribution of attacked shoots. and reference to Table III will show that the percentage infestation of shoots in the same stage of growth was approximately uniform. The primary shoots of the second sowing formed an exception, but here the coefficient of variation was extremely high (79.5) as compared with plots 3 and 26 (16.6 and 38.2 respectively). On the whole, the distribution may be considered to be approximately uniform and therefore that the data obtained from each group of the Victory plots may be considered together

Table IV.

*Coefficients of Correlation between actual and percentage infestations.*A. *First sowing.*

1. Primary shoots:
  - (a) Whole series ... .. +0.96 ±0.01
  - (b) Omitting Tam Finlay and Kent Berlie ... .. +0.89 ±0.02
  - (c) Omitting Supreme, Sandy, Tam Finlay, Kent Berlie and *Avena fatua* ... .. +0.90 ±0.02
2. Total shoots:
  - (a) Whole series ... .. +0.78 ±0.04
  - (b) Cases where actual numbers of shoots infested reached 80 or less ... .. +0.72 ±0.06
  - (c) Cases where actual numbers of shoots infested reached 60 or less ... .. +0.64 ±0.08

B. *Second sowing.*

1. Primary shoots:
  - (a) Whole series ... .. +0.87 ±0.03
  - (b) Omitting Supreme and *Avena fatua* ... .. +0.94 ±0.01
2. Total shoots:
  - (a) Whole series ... .. +0.74 ±0.05
  - (b) Cases where actual numbers of shoots infested reached 200 or less ... .. +0.70 ±0.07
  - (c) Cases where actual numbers of shoots infested reached 175 or less ... .. +0.86 ±0.04

C. *Percentage infestations of the different series.*

1. Same series, primary and total shoots:
  - (a) First sowing ... .. +0.84 ±0.03
  - (b) Second sowing ... .. +0.95 ±0.01
2. Comparative series, first and second sowings:
  - (a) Primary shoots ... .. +0.49 ±0.09
  - (b) Total shoots ... .. +0.63 ±0.07

to provide standards of comparison for the other varieties. These results are shown at the bottom of Table III.

It now remains to determine the significance of the differences, which is readily determined in any one case by calculating the standard error of the difference ( $E$ ) from the standard errors of the observed percentage infestations ( $E = \sqrt{E_1^2 + E_2^2}$ ) and dividing the observed difference by this figure. In this way, a measure of significance is obtained which has great value in determining the degree of reliance which may be placed on such observed differences. When the measure of significance is of the order 2.57, then the chances are 100 to 1 in favour of the result. For this work a standard of 100 to 1 has been adopted, because it is of the utmost importance that the existence of a difference should be firmly established before varieties showing desirable characters should be utilised for breeding purposes. By such means also it should be possible to establish an order of merit, based on the evidence provided by each series. The data recorded in Table III have been subjected to statistical analysis, to

determine the significance of the observed differences, where such differences appear to be of some weight, and the results of such analyses are shown in Tables V to VIII.

Table V.

*First sowing, primary shoots.*

Varieties differing from Victory by more than 10 %.

Ref. no.	Variety	Difference between percentage infestations	Standard error of difference	Measure of significance
34	<i>Avena fatua</i>	+17.7	7.5	2.37
33	Kent Berlie	+16.2	5.7	2.85
28	Supreme	+15.9	7.5	2.13
32	Tam Finlay	+11.1	4.7	2.38
35	Victory*	—	—	—

\* Variety with which the above have been compared (infestation 4.1 %).

*First sowing, primary shoots.*

Although Chart V shows that there were sufficient larvae present to attack the susceptible primary shoots, there were apparently very few susceptible shoots present, as these shoots had reached the fourth-leaf stage by the beginning of the prevalence period of the fly. The majority of the varieties differed from Victory by less than 10 per cent. and have therefore been omitted from Table V. Of this series, therefore, Kent Berlie was the only variety definitely more susceptible to attack than Victory, although Tam Finlay and *Avena fatua* nearly approach the necessary standard.

On the other hand, Golden Rain, the cross between Lochows and Victory, Lüneburger Kley, White Yeoman and Mesdag escaped attack on the primary shoots altogether, but cannot be said to be of a more desirable type than Victory, from the point of view of direct resistance, as judged by the evidence afforded by this particular series.

*First sowing, total shoots.*

Here the complication of tillering is introduced and direct plus some indirect resistance is really being measured. Only those varieties which differed from Victory by more than 5 per cent. have been considered in the above synopsis. In this case, when the total numbers of shoots produced on the plots were considered, Kent Berlie, Sandy, King, *Avena fatua*, Tam Finlay and Supreme proved to be definitely less resistant than Victory. Among the twelve varieties which were significantly less susceptible to attack than Victory oats, differences of the order of 5 per cent.

Table VI.

*First sowing, total shoots.*

Varieties differing from Victory by more than 5%.

Ref. no.	Variety	Difference between percentage infestations	Standard error of difference	Measure of significance
33	Kent Berlie	+ 18.1	2.7	6.72
31	Sandy	+ 15.3	2.7	5.58
1	King	+ 11.7	3.2	3.68
34	<i>Avena fatua</i>	+ 11.4	3.1	3.64
32	Tam Finlay	+ 10.6	2.1	4.93
28	Supreme	+ 8.6	3.6	2.42
36	Victory*	—	—	—
5	Golden Rain II	- 5.1	2.2	2.30
16	Gophers	- 5.1	2.2	2.37
19	Hede	- 5.4	1.9	2.83
20	Summer	- 6.7	2.2	2.98
17	Kytö	8.4	2.1	3.98
10	Leutewitzer	- 9.6	2.3	4.20
18	Spet	10.7	1.9	5.54
14	White Yeoman	- 11.4	2.1	5.48
15	Dala	- 11.8	2.1	5.76
8	Lochows	- 11.9	1.9	6.40
7	Lochows × Victory	- 12.2	1.8	6.87
9	Lüneburger Kley	- 13.5	1.7	7.82

\* Variety with which above have been compared (infestation 18.5 %).

have significance and therefore these particular varieties may be placed in an order of merit, those showing a percentage difference in infestation below - 7 per cent. forming a group definitely less resistant than those composing the group infested to the extent of - 12 per cent. and above. But it is not possible here to distinguish between Spet and Lüneburger Kley.

*Second sowing, primary shoots.*

Here the populations are approximately uniform in character. Within this series, in addition to those varieties which only differ from Victory by 5 per cent. or less, it is not possible to differentiate between Victory and *Avena fatua*, Star, Kent Berlie, Black Tartar, Mesdag, Black Bell II and Tam Finlay, in spite of the extent of some of the observed differences. The remaining fifteen varieties were, however, significantly more resistant than Victory. Considering this group alone, detailed analysis indicates that differences in percentage infestation of the order of 10 per cent. and above are significant, therefore those varieties showing a difference of - 21.5 per cent. and above, namely Hede, Spet and Summer, were

Table VII.

*Second sowing, primary shoots.*

Ref. no.	Variety	Difference between percentage infestations	Standard error of difference	Measure of significance
34	<i>Avena fatua</i>	+ 12.8	5.9	2.16
2	Star	+ 11.7	4.2	1.59
33	Kent Berlie	+ 10.4	6.1	1.71
27	Black Tartar	+ 6.8	4.6	1.49
30	Mesdag	+ 5.3	4.6	1.15
35	Victory*	—	—	—
21	Black Bell 11	- 9.2	3.9	2.38
14	White Yeoman	10.3	3.7	2.78
24	Argus	10.3	3.9	2.62
32	Tam Finlay	10.3	5.2	1.96
16	Gophers	11.6	3.7	3.16
9	Lüneburger Kley	14.3	3.6	3.94
25	Roslags	15.8	3.4	4.64
17	Kytö	17.2	3.4	5.08
22	Engelbrekt	17.4	3.3	5.20
8	Lochows	17.5	3.4	5.11
23	Great Mogul	17.7	3.3	5.28
10	Leutewitzer	19.0	3.6	5.22
7	Lochows × Victory	19.7	3.3	6.07
15	Dala	20.3	3.4	6.00
19	Hede	- 26.3	2.8	9.38
18	Spet	26.5	2.8	9.32
20	Summer	27.7	2.6	10.50

\* Variety with which the others have been compared (infestation 31.5 %).

definitely more directly resistant than those showing a difference of only — 11.5 or less, namely White Yeoman, Argus, Gophers and Lüneburger Kley. The comparison of data from such population of primary shoots has greater significance than when the total shoots are considered.

*Second sowing, total shoots.*

Again only those varieties which differed from Victory by more than 5 per cent. have been tabulated. Kent Berlie and Supreme alone maintained their position as being less resistant than Victory. In addition, this series indicates that Star, Mesdag and Black Tartar may under certain conditions suffer more heavily than Victory. In consequence of the population of shoots included being much greater in this series than in the previous series, the errors were much reduced and examination of those varieties showing greater resistance than Victory indicates that

Table VIII.

*Second sowing, total shoots.*

Ref. no.	Variety	Difference between percentage infestations	Standard error of difference	Measure of significance
33	Kent Berlie	+ 8.8	2.1	4.08
28	Supreme	+ 8.1	3.4	2.42
2	Star	+ 7.8	2.3	3.37
30	Mesdag	+ 6.7	2.6	2.57
27	Black Tartar	+ 6.6	2.4	2.78
35	Victory*	—	—	—
16	Gophers	- 8.2	2.0	4.08
17	Kytö	- 8.5	2.2	3.87
8	Lochows	- 9.0	2.2	4.17
9	Lüneburger Kley	- 9.3	2.1	4.38
14	White Yeoman	- 9.3	2.1	4.32
15	Dala	- 10.0	2.2	4.63
23	Great Mogul	- 11.5	2.0	5.61
25	Roslags	- 12.1	2.0	6.02
10	Leutewitzer	- 12.9	2.2	5.88
22	Engelbrekt	- 12.9	2.0	6.32
7	Lochows × Victory	- 14.4	2.0	7.20
18	Spet	- 20.3	1.8	11.20
19	Hede	- 21.4	1.6	13.05
20	Summer	- 22.5	1.4	15.65

\* Variety with which above have been compared (infestation 33.7 %).

here differences in percentage infestation of the order of 6 per cent. and above are significant. Therefore, in this series Spet, Hede and Summer proved definitely to be more resistant to attack than the remainder of the series.

#### (c) INTERPRETATION OF DATA.

The evidence derived from the study of the extent of the attack on the primary shoots of the later sowing indicated that Hede, Spet and Summer are varieties likely to be most suitable for breeding purposes. The varieties which appear in each of the three series (the first series of primary shoots providing no good evidence), and which therefore may be said to be more resistant than Victory, are Lochows × Victory, Lochows, Lüneburger Kley, Leutewitzer, White Yeoman, Dala, Kytö, Sept, Hede and Summer. The evaluations of differences in percentage infestation, observed to exist between these varieties and Victory, for the two series including all the shoots, are not of the same order in the case of each variety, therefore these values have been combined to obtain a weighted mean figure for each variety, for comparison with the results obtained from the primary



shoots of the later sowing. The results of these combinations are shown in Table IX.

Table IX.

*Weighted mean differences in percentage infestations, total shoots.*

Variety		Difference between percentage infestation, compared with Victory	Standard error of difference, squared	Weighted mean difference in percentage infestation	Standard error of weighted mean difference
Kytö	(a)	- 8.4	4.46	- 8.5	1.54
	(b)	- 8.5	4.77		
White Yeoman	(a)	- 11.4	4.33	- 10.3	1.49
	(b)	9.3	4.64		
Lochows	(a)	- 11.9	3.45	- 10.6	1.41
	(b)	- 9.0	4.67		
Dala	(a)	- 11.8	4.20	- 11.0	1.49
	(b)	10.0	4.71		
Leutewitzer	(a)	- 9.6	5.21	11.3	1.58
	(b)	12.9	4.80		
Lüneburger Kley	(a)	13.5	3.06	- 11.8	1.35
	(b)	- 9.3	4.47		
Lochows × Victory	(a)	12.2	3.15	13.3	1.32
	(b)	14.4	4.01		
Hede	(a)	5.4	3.61	14.6	1.24
	(b)	- 21.4	2.68		
Spet	(a)	10.7	3.72	15.8	1.32
	(b)	20.3	3.32		
Summer	(a)	6.7	5.01	- 17.6	1.20
	(b)	- 22.5	2.07		

(a) = first sowing; (b) = second sowing.

With these weighted means a difference of 5 per cent. is significant, that is, varieties giving values of differences above 15.5 per cent. are sharply differentiated from varieties giving values below 10.5 per cent. Therefore Summer and Spet are significantly most resistant on the evidence of both series and also are most directly resistant as shown by the series comprising primary shoots alone. The varieties occupying the intermediate positions may be associated with either of the extreme types, but the character of the available data precludes any definite expression of opinion as to their comparative resistant powers, amongst themselves. All the varieties shown in Table IX are of course more resistant to attack than Victory oats.

#### (d) OTHER OBSERVATIONS ON VARIETAL RESISTANCE.

Certain other observations, on a much less extensive scale, were made on some of the varieties used in the previous experiments, also on different varieties, to determine firstly whether different conditions would exert

much influence on differences in resistance, and secondly, whether any yield data obtained from the previous experiments would be confirmed by experimentation on a somewhat larger scale. These data are recorded in the following sections, under the headings (1) large comparative trial, and (2) data from observation plots. In a third section a Lochows  $\times$  Victory crossing, actually bred for commercial purposes, is compared with Victory and Lochows from the standpoint of resistance.

### 1. *Large comparative trial.*

Certain of the varieties, namely Victory, Golden Rain, Black Bell II, Lochows Yellow, Hede, Gophers and Lochows  $\times$  Victory, used in the previous experiments were sown on plots measuring  $1 \times 10$  metres, at a drill spacing of 12 cm., for the purpose of more accurately measuring the yield after recovery from attack, to check any data obtained from the later sowings made in the previous experiment. Sowing was carried out with a Pragner drill, having four coulter, at a field rate of sowing (180–200 kg. per hectare). Five replications of each series were made, but Golden Rain and Gophers were omitted from the second series owing to lack of suitable ground. By June 22nd the first sown series was in the late three-leaf and early four-leaf stages and the plants were tillering, while the later sown series was showing through. By June 29th the first series was in the late four- and early five-leaf stages and the later sown series in the early two-leaf stage. The first series of plants therefore passed through its period of susceptibility when the flies were in their maximum prevalence period, but the second series, although equally susceptible, was exposed only to a decreasing population. Samples, comprising the plants in 1 metre length of drill, from one drill of each variety

Table X.

*Large comparative trial. Percentage infestation with standard error.*

Variety in order of sowing	Percentage infestation with standard error		Difference in percentage infestations compared with Victory	
	First sowing	Second sowing	First sowing	Second sowing
Victory	34.7 $\pm$ 2.2	19.4 $\pm$ 2.3	—	—
Golden Rain	34.6 $\pm$ 2.3	Not sown	- 0.1 $\pm$ 3.2	—
Black Bell II	22.0 $\pm$ 2.1	7.7 $\pm$ 1.5	- 12.7 $\pm$ 3.0	- 11.7 $\pm$ 4.3
Lochows	26.6 $\pm$ 2.0	8.7 $\pm$ 1.7	8.1 $\pm$ 2.9	- 10.7 $\pm$ 3.7
Hede	11.9 $\pm$ 1.3	11.5 $\pm$ 1.8	- 22.6 $\pm$ 2.5	- 7.9 $\pm$ 2.7
Gophers	30.8 $\pm$ 2.2	Not sown	- 3.9 $\pm$ 3.1	—
Victory $\times$ Lochows	22.7 $\pm$ 2.0	9.2 $\pm$ 1.6	- 12.0 $\pm$ 2.9	- 10.2 $\pm$ 3.7

throughout the series, were taken on July 11th and 12th and analysed, the results being recorded in Table X.

The heavier infestation of the first sowing is explained by the fact that only this series was exposed to any considerable number of flies. It is interesting to note that both Golden Rain and Gophers again were not any more resistant than Victory, while Bell II, Lochows, Hede and Lochows  $\times$  Victory proved to be superior in each series. Of these four varieties, Hede was significantly more resistant than the other three in the first series but it was not differentiated in the second series.

It is somewhat doubtful whether the yield data from these experiments will have any value as the crops suffered very much from moisture during maturation.

## 2. *Data from observation plots.*

Large numbers of different varieties of oats are sown annually at Svalöv for observation purposes only, and examination of some of these varieties was rendered possible by the courtesy of Dr Åkerman. These varieties were sown on metre plots at the normal board spacing and they reached the four-leaf stage by June 16th, their susceptible stages therefore coinciding with the increasing prevalence of the fly. The plots were sampled on July 7th in two places, namely, at the extreme edges of the plots, the minimum, mean and maximum numbers of plants and shoots per sample being 15, 24, 30 and 47, 106, 167 respectively. The sampling was necessarily restricted by the fact that the material was required for other purposes and the results should only be considered in conjunction with those obtained from other sources. In estimating the infestation the samples have been taken together and the standard errors worked out on the basis of the number of shoots per sample. These data are shown in Table XI.

All these varieties except Nidar were actually less infested than Victory, but, as may be seen by reference to Table XI, the observed differences were not significant in the case of ten of them. Of the fifteen varieties, significantly less infested than Victory, twelve were less infested to the extent of 10 per cent. and above, but none of these differences, which range from  $-12.0$  to  $-19.7$  per cent., were significant, therefore these varieties, which include Lochows  $\times$  Victory, Lochows, Lochows  $\times$  Golden Rain and Roslags, cannot be differentiated amongst themselves on this evidence. The general trend of these data confirmed the results of the previous variety trial. Only five were infested to the extent of 5 per cent. above Lochows  $\times$  Victory, the varieties from Norrbottens

Table XI.

*Observation plots.*

Swedish ref. no.	Variety	Percentage infestation with standard error	Difference in percentage infestation compared with Victory	Standard error of difference	Measure of signifi- cance
861	Victory	27.9 ± 2.1	—	—	—
868	Abeds Nova × Victory	23.7 ± 2.7	- 4.2	3.4	1.22
876	Lochows × Victory	9.2 ± 1.8	- 18.7	2.8	6.70
877	Another line of above	10.4 ± 2.1	- 17.5	3.0	5.84
878	Lochows × Golden Rain	8.2 ± 1.8	- 19.7	2.8	7.12
879	Lochows Yellow	13.8 ± 2.4	- 14.1	3.2	4.41
882.9	Yellow Flanders	14.9 ± 2.7	- 13.0	3.4	3.70
882.10	French Yellow Giant	9.8 ± 2.4	- 18.1	3.2	5.69
884.3	Walstedt (Bell II)	18.9 ± 2.9	9.0	3.6	2.50
884.6	Another line of above	19.6 ± 2.7	8.3	3.4	2.44
884.7	" " "	15.9 ± 2.3	- 12.0	3.1	3.86
884.9	Engelbrekt	14.2 ± 2.3	13.7	3.1	4.44
884.13	Roslag × Bell II	8.8 ± 1.9	- 19.1	2.8	6.72
884.15	Argus	22.5 ± 3.3	- 5.4	3.9	1.37
884.16	Bell III × Golden Rain	11.7 ± 2.0	- 16.2	2.9	5.52
884.22	Roslags	12.0 ± 2.0	- 15.9	2.9	5.47
884.25	Wisingö	18.3 ± 2.5	- 9.6	3.3	2.92
886.5	Ex Angermanlant	15.5 ± 2.3	- 12.4	3.1	3.95
886.6	Thor	21.3 ± 3.6	- 6.6	4.2	1.57
886.9	Nidar	28.8 ± 3.8	+ 0.9	4.3	0.21
888.9	Mesdag	18.9 ± 3.5	- 9.0	4.0	2.22
888.10	Norrbottnens	25.3 ± 3.2	- 2.6	3.8	0.68
888.11	Another line of same	18.3 ± 2.8	- 9.6	3.5	2.74
892.1	Mulga (N.S.W.)	21.9 ± 3.2	- 6.0	3.8	1.58
895.1	Joanette	24.7 ± 2.4	- 3.2	3.2	1.00

and Wisingö alone showing significantly less resistance than Lochows × Victory.

### 3. *A Lochows × Victory crossing and its results.*

The Director of one of the Svalöv sub-stations made the crossing between Lochows, the common German yellow oat and Victory, and found one or more of the lines therefrom to give promise of higher yielding capacity than Victory. Hence it was brought under observation at Svalöv, found to maintain its yielding capacity and is now being multiplied for commercial purposes, probably reaching the market in two years time. Dr Åkerman thought that, by visual observation, he could distinguish a difference in resistance in spring between Lochows and Victory, and it was a matter of considerable interest to determine whether the

cross Lochows  $\times$  Victory would inherit the resistance of Lochows, if the latter were proved to be resistant also.

Five sets of data relating to the resistance of these three varieties have been examined and a weighted mean difference in percentage attack, as compared with Victory, obtained for both Lochows and the cross, Lochows  $\times$  Victory, from four of the sets, in which the estimation of the percentage infestation has been based on the total numbers of shoots present on the plots. The collected data are shown in Table XII, except for the data provided by the primary shoots of the second sowing in the variety trial, which indicated a difference in percentage infestation, as compared with Victory, of the order of  $-17.5 \pm 3.4$  for Lochows Yellow and  $-19.7 \pm 3.3$  for the cross Lochows  $\times$  Victory.

Table XII.

*Difference between percentage infestations as compared with Victory.*

Variety	Variety trial		Com- parative trial total shoots	Observa- tion plots total shoots
	First sowing total shoots	Second sowing total shoots		
Lochows Yellow	$-11.9 \pm 1.9$	$-9.0 \pm 2.2$	$-8.1 \pm 2.9$	$-14.1 \pm 3.2$
Lochows Yellow $\times$ Victory	$-12.2 \pm 1.8$	$-14.4 \pm 2.0$	$-12.0 \pm 2.9$	$-17.5 \pm 3.0$

The weighted mean difference in percentage infestation was  $-10.7 \pm 1.2$  for Lochows Yellow and  $-13.6 \pm 1.1$  for Lochows  $\times$  Victory, the significance of the difference between these two being of the order of 2.25 ( $D = 2.9 \pm 1.29$ ). The result is of interest because it shows that valuable economic results may be the outcome of such investigations.

#### VIII. UTILISATION OF OBSERVATIONS ON RESISTANCE.

The object of the previous experimentation was of course to discover the varieties most resistant, in both senses of the term, to the attack of the frit fly. Certain information having been acquired the next step was to utilise this information for building up a heavy yielding resistant variety, because the heavy yielding varieties themselves unfortunately occupy low positions in the scale of resistance. The work of crossing had to be carried out immediately after the analysis of the shoots, namely about July 20th onwards, and in consequence it was possible only to glean a very general idea, from the mass of recorded but unanalysed data, as to which were the most resistant varieties. As will be seen by reference to Table XIII ten of the varieties showing some evidence of superiority over Victory were crossed with Victory, Golden Rain II and Star.

Table XIII.

*Varieties used for breeding, showing numbers of panicles, each of which carried 20–25 spikelets, utilised for crossings.*

	Victory	Golden Rain II	Star	Dala	Ligowo
Lochows × Victory	5	5	.	.	.
Lochows	5	4	5	.	.
Lüneburger	5	.	.	.	.
Hede	12	15	5	3	.
Spet	13	10	5	.	4
Leutewitzer	4	.	4	.	5
Dala	5	.	5	.	.
Summer	4	.	2	.	.
Roslags	5	.	5	.	.
Kytö	.	5	5	.	.

This breeding work is at present purely empirical, no evidence whatever being available as to what characters may be associated with resistance. From previous work it seems likely that the fourth-leaf stage is the critical stage of the growth period of the plant, when the rapid elongation of the internode may bear the growing point out of reach of the minute larva or when the renewal of growth after tiller formation may induce unfavourable conditions for the larva, due to biochemical changes in the sap itself. Changes or differences in such characters or in physical characters of the tissues may account for the differences in resistance shown by the different varieties. In the absence of any such illuminating facts and without much precise information as to the reality of observed differences, it was only possible to make crosses somewhat at hazard and hope that some of the progeny would show both desirable characters, namely high resistance and high yielding capability. Hede and Spet were used for making numerous crossings with Victory and Golden Rain II because (1) of the notorious difficulty associated with seed-setting in oats after artificial pollination, 2 or 3 per cent. being the normal order of success in Sweden, and (2) of the desirability of attaining some success with these two varieties. Without the able and willing assistance of the technical staff of Dr Åkerman it would not have been possible to complete this work, because it involved the fertilisation of approximately 3500 flowers, carried on about 150 panicles. The progeny of these crosses were sown as early as possible in the spring of 1928, to obtain from them the maximum numbers of seed. The  $F_2$  generations will then be sown late in the spring of 1929, and after natural selection by the fly has eliminated the

less resistant types, desirable types will be selected for multiplication for further examination by yield trials.

#### IX. APPENDIX. A SECOND ANALYTICAL METHOD APPLIED TO THE DATA.

As this analytical method may not be considered applicable by some, the data have also been analysed by the method which utilises the average value of the variance, obtained by considering all possible pairs of varieties(8). The standard error of the mean difference in percentage infestation between any two varieties, for each of the series except the primary shoots of the first sowing, is shown in the following table:

	Standard error of mean difference	Standard error $\times 2.57$
First sowing, primary shoots	Insufficient data	—
First sowing, total shoots	4.08	10.5
Second sowing, primary shoots	6.38	16.4
Second sowing, total shoots	4.77	12.3

The standard error, multiplied by the figure 2.57, gives a value which may be used to differentiate between any two records in each series tabulated in Table III. Percentage infestations differing by more than these values, within the respective series, are significantly different. This method of presentation, therefore, makes it very simple for anyone to determine the comparative position of any variety. In general, of course, the two methods give equivalent results, but in this case the differentiation is not so fine.

#### REFERENCES.

- (1) CUNLIFFE, N. (1921). Preliminary observations on the habits of *Oscinella frit* Linn. *Ann. App. Biol.* VIII, No. 2, 106-134.
- (2) — (1924). Further observations on the prevalence and habits of *Oscinella frit* Linn. *Ibid.* XI, No. 1, 54-72.
- (3) — (1925). *Oscinella frit* Linn. A note on the seasonal regularity of the maximum prevalence periods of the fly in the field. *Ibid.* XII, No. 4, 527-528.
- (4) — (1928). Studies on *Oscinella frit* Linn. Observations on infestation and yield, susceptibility to infestation, recovery power, the influence of variety on the rate of growth of the primary shoot of the oat and the reaction to manurial treatment. *Ibid.* XV, No. 3, 473-487.
- (5) CUNLIFFE, N. and FRYER, J. C. F. (1924). *Oscinella frit* Linn. An investigation to determine how far varietal differences may influence infestation of the oat plant. *Ibid.* XI, Nos. 3-4, 465-481.

- (6) CUNLIFFE, N. and FRYER, J. C. F. (1925). Studies on *Oscinella frit* Linn. Supplementary data on the relation between varietal differences of oat plants and susceptibility to infestation. *Ibid.* xii, No. 4, 508-515.
- (7) CUNLIFFE, N., FRYER, J. C. F. and GIBSON, G. W. (1925). The correlation between stage of growth of stem and susceptibility to infestation. *Ibid.* xii, No. 4, 516-526.
- (8) ENGLEADOW, F. L. and YULE, G. U. (1927). *The principles and practice of yield trials*. Empire Cotton Growing Corporation, London.
- (9) FRYER, J. C. F. and COLLIN, J. E. (1924). Certain aspects of the damage to oats by the frit fly. *Ann. App. Biol.* xi, Nos. 3-4, 448-464.
- (10) PETHERBRIDGE, F. R. (1924). The common cause of failure of spring oats—frit fly. *Journ. Mins. Agric.* xxxi, 925.

(Received June 1st, 1928.)



## NOTE ON THE GROWTH OF YOUNG MICE SUCKLED BY RATS

By A. S. PARKES, D.Sc. (*Beit Memorial Research Fellow*).  
(*From the Department of Physiology and Biochemistry, University  
College, London.*)

IN a previous paper (Parkes<sup>(1)</sup>) it was shown that the growth of young mice during the suckling period was inversely proportional to the number suckling, and all the evidence suggested that this differential growth probably depended on the fact that each member of a large litter received less nutriment than one from a small litter. The growth curves of the individuals of the small litters (one and two) were extremely steep, and resulted in relatively enormous animals at weaning time.

In view of these results it was clear that it would be of interest to provide an unlimited milk supply for even a large litter and thus to ascertain:

(a) Whether the explanation put forward previously relating to differential nutrition could be substantiated by showing that the difference in the growth curves of large and small litters disappeared under conditions of unlimited nutrition.

(b) What ultimate degree of steepness in the growth curve could be attained by unlimited nutrition.

It was thought possible that such conditions of practically unlimited nutrition could be obtained by foster-mothering young mice on to lactating rats. The lactating rat produces enough milk to allow of the growth of five to seven young rats from 5.0 gm. at birth to 30 gm. at 3 weeks, *i.e.* to allow of the production of 100 to 150 gm. live weight. This amount would enable mice to reach adult weight by weaning time, and would represent, therefore, practically an unlimited nutrition for the smaller animal. This idea of rearing young mice on lactating rats was found in practice to be feasible, but the manœuvre required much care in selecting docile rats, etc., and even when the mice were not eaten, many difficulties arose, such as the weight of the rat when lying on the young mice and the large size of the nipples. In spite of all this, however, sufficient success was attained to make it possible to collect the following data.

Seven litters of mice in all were reared solely or partially on rats. The table shows the age at which the transfer to the rat was made, and the size of the litter, together with the average daily weight.

*Growth of young mice suckled by rats.*

No. of litter	GM 1	GM 2	GM 3	GM 4	GM 5	GM 6	GM 7	{Normal for mice	
Size of litter	5	2	3	4	5	7	6	—	
Day put on to rat	10	At birth	10	7	3	9	2	—	
Average weight in gms. per days old	0	—	2.1	—	—	1.9	1.6	1.8	1.41
	1	—	2.2	—	—	2.2	2.1	2.2	1.64
	2	—	2.5	—	—	2.4	2.3	2.5	1.90
	3	—	3.5	—	—	2.8	2.7	2.9	2.20
	4	—	4.0	—	—	3.0 (4)	3.0	3.5	2.53
	5	—	5.0	—	—	3.4	3.4	4.2	2.85
	6	—	5.7	—	—	3.9	3.7	4.8	3.19
	7	—	6.5	—	4.1	4.4	4.1	5.3	3.54
	8	—	7.2	—	4.8	5.0	4.4	5.8	3.86
	9	—	8.2	—	5.5	5.6	4.6	6.4	4.15
	10	4.6	9.0	6.3	6.2	6.4	5.2	7.2	4.43
	11	5.4	9.7	6.8	7.1	6.9	5.6	7.8	4.66
	12	6.4	10.5	7.4	8.0	7.4	6.2	8.4	4.85
	13	7.2	11.5	7.8	8.5	8.0	6.5	9.0	5.04
	14	7.7	12.2	8.3	9.0	8.5	6.9	*	5.20
	15	8.2	12.8	9.0	9.6	*	7.3	—	5.33
	16	9.0	13.5	9.4	10.1	—	7.5	—	5.48
	17	9.4	14.0	9.9	10.7	—	7.7	—	5.71
	18	9.9	14.7	10.3	11.3	—	8.0	—	6.00
	19	10.9	15.5	10.8	12.0	—	8.3	—	6.35
	20	11.3	16.1	11.5	12.7	—	8.5	—	6.72
	21	11.8	17.0	12.2	13.5	—	8.9	—	7.14

\* Overlaid by rat.

From this table it will be seen that where only two young were put to the rat the phenomenal average weight of 17.0 gm. was attained by 21 days old. This increase in weight is nearly double that found in litters of two suckled on mice (Parkes(1)), and some idea of its magnitude can be obtained by considering that anything between 50 and 100 c.c. of milk each must have been consumed in the 3 weeks. This great increase in size, however, did not cause a corresponding increase in development (the eyes, for instance, opened at the normal time—13 days old), and the animals at 3 weeks old were practically immobile owing to the failure of the immature frame to cope with a weight almost equal to that of the adult animal.

Owing to their increased number and to being put to the rats later the other litters failed to show quite as surprising a growth as was found in GM 2. but in every instance the growth found was far in excess of that normal for the size of litter in question. The correlation between

number suckling and growth rate is not found in these mice suckled by rats.

From these results it is possible to conclude:

(a) That the variation in the growth of the various sizes of litter in the normal mouse is purely a question of differential nutrition.

(b) Under conditions of unlimited nutrition the growth of young mice may proceed to a degree which is both unusual and unhealthy.

#### REFERENCE.

- (1) PARKES (1926). The growth of young mice according to size of litter. *Ann. App. Biol.* XIII.

(Received July 26th, 1928.)

## REVIEWS

*Filterable Viruses.* By T. M. RIVERS. London: Baillière, Tindall and Cox, 1928. Pp. ix + 428. 15 Plates and 26 Figures. Royal 8vo. 34s. net.

Virus diseases are known to occur in man and other mammals, in birds, insects, plants, even, it is probable, in bacteria. It is no small labour merely to discover all the papers dealing with a subject whose matter covers so large a field and is so distributed, and the literature is now so vast that even the specialist can hardly keep in touch with all its ramifications. Yet a stray fact definitely established in any one field, however remote, may at any time throw a light upon obscurities in all the others. A book, therefore, which brings together in a general survey the information accumulated in regions so diverse is sure of a welcome. No general survey, of course, can be expected to give a complete analysis of all the work that has been done on virus diseases, and even in this volume of over 400 pages the treatment has been simplified by the selection of representative diseases within the various groups. Thus we have separate chapters on the virus diseases of man as exemplified by poliomyelitis, of mammals as exemplified by foot-and-mouth disease, of birds by fowl-pox, followed by chapters on the virus diseases of insects, of plants and of bacteria. With the possible exception of bacteriophagy, these diseases would be generally accepted as genuine virus diseases, but no satisfactory answer can be given to the question "what *is* a virus disease"? There are at present no definite criteria by which to decide whether any particular disease is or is not due to virus. Even the filterability of the causal agent—and the title of this book is *Filterable Viruses*—does not sharply differentiate the group, because on the one hand this character is shared by some bacteria, vibrios, spirochetes and protozoa, and on the other in some admittedly virus diseases, *e.g.* chicken-pox, no filtration experiments are recorded, and in others, *e.g.* vaccinia, the agent is either not filterable at all or filterable only with the greatest difficulty.

The volume begins with a chapter on "Some General Aspects of Filterable Viruses" by Dr Rivers. In this chapter (which has already appeared in the *Journal of Bacteriology* in substantially its present form) the author enumerates the outstanding problems which the study of virus diseases has raised, immunity, size and filterability of the agents, specificity, influence on cells and the like, summarises briefly (too briefly, we think) the information available under each head, suggests some lines for future work, and concludes with a summary of seven lines. In our opinion this chapter might well have been expanded considerably. Dr Rivers has restricted himself very largely to a statement of the various conflicting views and a conclusion that this or that question has not been satisfactorily settled. We should have welcomed a discussion on broader lines, especially since the summary here given is already in print and available for most workers. There is a number of aspects, barely or not at all touched upon, that might well have received consideration, *e.g.* the consequences that must follow from an intracellular habit of life as contrasted with the extracellular habit of most bacteria, the existence of toxins, the possibility of origin *de novo* (Rous's tumour).

This first general chapter is followed by one on filters and filtration by Prof. Stuart Mudd, a very useful chapter, and a salutary because an astounding quantity of nonsense has been deduced from observations on filtration. This is a good chapter. No mention is made of d'Herelle's method of sterilising membrane filters. A very interesting discussion by Carrel of tissue-culture in the study of viruses follows; and after this there is a cautious chapter by Dr Cowdry on intracellular pathology with some excellent illustrations of cell-inclusions, and a judicious discussion of the possibility of drawing conclusions from the available data. Then follow the chapters already

mentioned on typical virus diseases. Each of these is by a different writer, of authority on the subject with which he deals. No doubt the specialist on each will find points to disagree with and statements he is not ready to accept; but each is well done and gives an excellent presentment of its subject.

On the whole a very useful book: sometimes very good and occasionally stimulating.

J. HENDERSON SMITH.

- (1) *Manual of Plant Diseases*. By F. D. HEALD. McGraw-Hill Publishing Co., Ltd., 1926. Pp. xiii + 891. 272 Text-figures. Price 35s. net.
- (2) *Principles of Plant Pathology*. By C. E. OWENS. New York: John Wiley and Sons, 1928. Pp. xii + 627. 222 Text-figures. Price 23s. 6d.
- (3) *Comparative Morphology of Fungi*. By E. A. GÄUMANN. Translated and revised by C. W. DODGE. McGraw-Hill Publishing Co., Ltd., 1928. Pp. xiv + 701. 406 Text-figures. Diagrams XLIII. Price 37s. 6d.

The past decade has been notable in plant pathology for the number of textbooks which have been published—in Germany a new edition of Sorauer and volumes by Höstermann and Noack, Riehm, Kirchner, Neger, Morstatt, Köck and Fulmek, Graebner, etc.; in France volumes by Marchal, Mangin, Nicolle and Magrou and new editions of Delacroix and Maublanc, Bourcart, etc.; in Sweden a new edition of Eriksson; in Spain a textbook by Gonzalez; in Czecho-Slovakia one by Smólak; in Russia volumes by Naoumoff and Bondarzew; in Japan treatises by Suematu and Ideta; in various parts of the British Empire books by van der Bijl, Cunningham, Butler, Nowell, Petch, Dade and Bunting, Bewley, Fryer, etc.; in the United States volumes by Stevens and Hall, Taubenhaus, Hesler and Whetzel, Chupp, Harshberger, Smith, Fawcett and Lee, Rankin, Mason, Anderson and Roth, etc., and the present authors Heald and Owens. Of the above some are general works, whilst others deal with one or other aspect of plant pathology but they are all alike in being of the nature of textbooks.

This fecundity is partly due to post-war reaction, but, even so, no science can reproduce so prolifically unless it has reached years of maturity. Plant pathology, which is so largely the child of academic botany, has as a matter of fact come of age. As a science it is showing increasing independence of its parent; founding its own professorial chairs, its own teaching schools, its own research institutes and its own committees of management. Although linked to botany by filial ties and common interests, yet it has its own life to live and its own contribution to make to knowledge and to human welfare. In America the separation has become almost complete; in England it is admitted almost less than in any other country and the parental authority of academic botany is still greatly in evidence. The present volumes may make these movements a little clearer to the older fashioned botanists and perhaps induce a more understanding and sympathetic attitude of mind to what is an inevitable course of development.

A second point of general interest arising out of these books and one that is ripe for discussion, especially in England, relates to the scope of plant pathology. Prof. Owens appends to his chapters lists of "Review Questions," and one of these asks "What is the difference between 'plant pathology' and 'mycology'?" Many students in this country do not recognise any difference and point to the fact that in England "plant pathologists" are in almost all cases officially termed "mycologists." On the other hand, a few years ago a distinguished American student wrote "Until very recent years plant pathology has been considered as simply a phase of botany or as applied mycology. A brief course in mycology masquerading under the name of plant pathology has in most cases sufficed to dispose of the subject. Even the so-called

plant pathologists of the present day are in large part only mycologists with little of the true phytopathologic point of view."

Heald answers the question by describing his book as "An attempt... to present a view of the whole field of plant pathology including environmental and virus diseases as well as those of bacterial and fungous origin, as it is felt that a book of restricted scope would perpetuate an erroneous notion which has been prevalent in recent years as to the real province of plant pathology." The latter he defines as "The consideration of all non-parasitic diseases, the virus diseases, all troubles due to the five groups of plant parasites, and in addition... those due to nematodes and also those of protozoan origin." The above sentences might equally well have been quoted from Owens' treatise.

Until plant pathology secures autonomy on this wide basis and takes rank with such independent disciplines as human and veterinary medicine, the science as a science will make little progress. The English titles of "Mycologist" and "Mycology" give a false idea of subordinate value and dependence, once perhaps true, but now erroneous and stultifying and subversive of the best interests of plant pathology. The equation of mycology with plant pathology has in addition a serious adverse influence on the study of mycology itself, for attention is deflected from pure research on the fungi to more economic ends. Mycology is the study of fungi *qua* fungi and plant pathology is the study of plant disease *qua* plant disease whatever its etiology, state or relations. Their viewpoints are fundamentally different and their only contact is that a certain considerable area of their provinces is held in common just as happens with veterinary medicine and physiology or human medicine and bacteriology. The difference between the sciences is well shown by a comparison of the volume by Gäumann and Dodge with those by Owens and Heald.

Gäumann and Dodge's book is an account of the structure and development of the fungi written to give understanding of these organisms as living things with intrinsic value and interest of their own, irrespective of any economic relationships they may show with other organisms, their inanimate environment or matters of human welfare. The fungi are studied for their own sake as one studies the algae or the pteridophytes.

The volumes by Heald and Owens are concerned with disease in plants; the nature of disease, the agencies which bring about disease, the losses to man caused by disease and the prevention and cure of disease. In Heald's book 58 pages are given to history and symptomatology, 175 pages to non-parasitic diseases, 55 pages to virus diseases, 63 pages to bacterial diseases, 19 pages to myxomycetous diseases, 440 pages to fungous diseases, 21 pages to phanerogamic diseases and 22 pages to nematode diseases. In brief about one-half the volume deals with diseases caused by fungi, but even here the diseased host is the centre of attention and not the fungus, the latter only being of interest in so far as its discussion contributes towards an understanding of the diseased state. In fact most of the pages dealing with fungous diseases are filled with descriptions of the history and distribution of the specific diseases, their symptoms, their effects and their economic importance; with discussions of predisposing factors, host relations, control measures, etc., the fungi themselves being little more than mentioned or briefly described.

The same construction appears in Owens' book. The first 157 pages are devoted to generalities concerning disease and its relation to environmental factors, 13 pages to myxomycetous diseases, 54 pages to bacterial diseases, 276 pages to fungous diseases, 16 pages to phanerogamic diseases, 20 pages to nematode diseases, 50 pages to virus diseases and 24 pages to various non-parasitic troubles. Here again the two-fifths of the book dealing with fungous diseases only treats of the fungi in so far as they are necessary to understand the diseased crop plant and most of the text deals with host symptoms, economic aspects and control measures.

If these books do anything to undermine the domination of plant pathology by academic mycology and so help to destroy what is clearly an untenable and obsolete position they will have done yeoman service.

A few words may be said in more direct appreciation and criticism of the volumes. For many years there has been great need of a major general textbook in English

on plant disease. Owens' volume was, apparently, first issued in mimeograph form in 1924 and has just been published in its present state, whilst Heald's book was issued in the winter of 1926-7.

*Principles of Plant Pathology* by Prof. Owens is essentially a textbook for the American undergraduate student and to each chapter are appended directions for laboratory study, "review questions" and extensive bibliographies in which the references are almost exclusively American. In fact, the whole volume is lacking in appreciation or even mention of non-American work. This is illustrated for example by the lists of textbooks and scientific journals given on p. 155. Only eleven journals are mentioned, of which eight are American, two are English—*Annals of Botany* and *Annals of Applied Biology*—and one is German—*Zeitschrift für Pflanzenkrankheiten*. Adequate comment is not easy. The actual diseases are on the whole well described and illustrated, and a student working conscientiously through the volume would emerge with a fairly sound knowledge of American plant diseases. If he then proceeded to a European laboratory to widen his experience and obtain a truer perspective he might become a sound plant pathologist.

The *Manual of Plant Diseases* by Prof. Heald is a textbook for students of better quality. The difference between the volumes by Owens and Heald is exemplified in the bibliographies appended to the chapters. Taking one at random, that on Downy Mildew of Grapes for example, Owens gives five name references—all American—whilst Heald gives fifteen, of which only two are American. The textbook by Owens is parochial in outlook, that by Heald much more cosmopolitan. In spite of minor detractions in Heald's book such as obvious misprints and somewhat trivial headings—"*Pondscum parasites*," etc., which look rather silly in a treatise of this calibre—the volume is a serious work of considerable value.

When Prof. Gaumann's *Vergleichende Morphologie der Pilze* was published in 1926 it was at once recognised as a work of outstanding importance. Since De Bary's volume 40 years previously no comprehensive treatise on this subject had appeared and the need was urgent, for in the interval the study of fungus morphology had made immense strides. The advance was almost entirely a filling in along lines largely foreshadowed by De Bary, and it has been concerned chiefly with the cytology of fungus reproduction, mycologists out-Freuding Freud in their obsession with sex. Gaumann brought together in a masterly way an amazing amount of data, his volume containing fine illustrations of a large number of fungi, and referring explicitly to species whose mere names comprise nearly all of 30 double-columned pages of Index.

The American edition is not a simple translation, for Prof. Dodge has incorporated the new literature appearing between 1925-7 and rewritten certain sections. The Ascomycetes and Basidiomycetes particularly have been reconsidered and one might perhaps instance the treatment of the Gasteromycetes or of the Laboulbeniales as striking improvements. But, *O tempora! O mores!* For 30 years students have observed almost with reverence the spermatia caught *flagrante delicto* on the trichogyne in Thaxter's figures of *Stigmatomyces Baeri* and now in a mere footnote we are told that these are not spermatia at all but only receptive prominences of the trichogyne itself!

In the American edition there are many minor alterations; the omission of the list of general books, correction of errors in synonymy, relegation of authorities to the Index and the assemblage of the several bibliographies in a single chapter of 40 pages at the end of the book. An unfortunate alteration is the introduction of numerous misprints. When one reads for example that the Ancylistaceae and the Pythiaceae are both "partially endosporaltic" it takes one a moment or two to decide that this is merely a misprint of "endoparasitic" and not still another technical term; and this kind of thing is too common in the volume.

Prof. Gaumann's original work is a little heavy going due to his strict adherence to technical terms, and the new edition is certainly no improvement—"The chiasmo-basidial Autobasidiomycetes have attained the same degree of development in this family as the stichobasidial Phragmobasidiomycetes in the Septobasidiaceae and the lepto-forms of the Uredinales"—and so on for 700 pages. Throughout, there is hardly any lightness of touch, and when, on page 288, the authors, speaking of the transitional

forms in the Sphaeriales remark, parenthetically, that these are "inconvenient for systematists" or on p. 455 describe the Agaricales as "of fatiguing regularity" one is inclined to smile at a huge joke.

The aim of the original work is well stated on p. 427 as "not to make easy the recognition and identification of an unknown fungus but to discuss fundamental questions of relationships and to point out gaps in our knowledge" and this purpose it fulfils admirably.

In the American edition Prof. Dodge has preserved many of the theoretical discussions of phylogeny, although he states in his preface that "it is impossible for me to agree with some of the conclusions." The whole structure of the work is based on certain phylogenetic concepts which are reviewed in the final chapter. The detailed applications of these are worked out for each section in turn and one must admire the way in which the original author kept in his mind the logical and processional sequence of values necessary for his constructions. The several moieties are crystallised out in numerous phylogenetic diagrams, and although one cannot here discuss these in detail one may perhaps express a personal opinion that the treatment is too didactic and exhibits a confidence in phylogenetic speculation which at times is a little astonishing. To describe the origin of any one genus of fungus from any other as "very evident" (p. 282) certainly gives one to pause. On a more general point one may wonder whether a two-dimensional diagram can in any way represent evolutionary sequence of a three-dimensional order.

Still, no book of this size and quality could please everyone and much of the fun of scientific research would disappear if we all lay down as lambs together. One can only be grateful to Prof. Dodge for his courage and skill in carrying out so onerous a task and assure him that botanical students throughout the English-speaking world will rise up and call him blessed.

WILLIAM B. BRIERLEY.

*Leaf-Mining Insects.* By JAMES G. NEEDHAM, STUART W. FROST and BEATRICE H. TOTHILL. London: Baillière, Tindall and Cox, 1928. Pp. viii + 351 and 91 Figures. Price 27s. net.

In their prefatory remarks the authors mention that the object of this book is threefold and aims at providing (1) a non-technical introduction to the general subject of leaf-mining insects; (2) an account of their biology sufficiently detailed to be of value to the ecologist; and (3) lists of miners and their host plants and of the principal papers on these insects.

Sufficient is known of leaf-miners to-day to show that their study affords many problems of interest to the general biologist and the specialist alike. With the present volume, along with Dr Martin Hering's excellent *Ökologie der blattminierende Insektenlarven*, the subject is brought well up to date with copious bibliographies.

Four orders of insects, viz. Lepidoptera, Coleoptera, Diptera and Hymenoptera, include leaf-mining species. In all cases it is the larva which betrays this habit which is undeveloped in the adults. In the first two chapters of the book before us there is an elementary general discussion of the types of leaf-mining larvae, the moulding effects of similarity of environment on larval forms of diverse groups, the characters of the mines themselves, the origin of the leaf-mining habit and the subject of host preference. A classification of mines is given and a general table for separating larvae of the four orders already mentioned. There are also brief remarks on collecting and rearing, and a short discussion of species of economic importance. Chapter III is concerned with Lepidopterous leaf-miners in general and the eight chapters which follow each deal with a separate sub-division of the order. The great development of the mining habit in the order is reflected in the fact that one-half of the book is devoted to these insects. The various grades in specialisation of the leaf-mining habit and the various modifications in the structure of the head-capsule and mouth-parts are of interest from the evolutionary standpoint, which the work of Trägårdh in Sweden has so excellently demonstrated.



Chapter XII deals with Coleoptera, an order wherein the leaf-mining habit is little developed. Chapter XIII is concerned with Hymenoptera where the habit is confined to the family Tenthredinidae and Chapter XIV deals with Diptera.

On the whole the book serves as a valuable introduction to the subject. It is especially useful to the field worker whether he be an ecologist or an economic entomologist, and will prove of very material assistance in the identification of the species met with. The lists of leaf-mining species (Chap. xv) and the hosts (Chap. xvi) are a special feature of the book, and the bibliography will serve as a useful guide for all who desire more detailed information.

Information on leaf-miners is now so extensive that it becomes impossible to deal, in a book of this compass, with every species concerning which some facts relating to their habits are available. The European forms are well treated by Hering, and the present volume is concerned more especially with the American species. We presume it is the authors' intention to thus limit their subject although it is not expressly mentioned in the Preface. If this conjecture be correct, it explains why some notable European species are neither mentioned nor listed. Its treatment is frankly elementary throughout and, having mastered this volume, the reader should be well prepared to study the work of Hering already alluded to. The book is well arranged, the printing good and the illustrations are, for the most part, clear and adequate. It is not free from typographical errors, but these are insufficient to detract materially from its value and it will find a useful place in North American entomological literature.

A. D. IMMS.

*Modern Biology.* By J. T. CUNNINGHAM. Pp. xii + 244. London: Kegan Paul, 1928. 10s. 6d. net.

A botanist cannot but feel a little aggrieved when a book dealing only with certain aspects of zoology and omitting almost all mention of plant life, is entitled *Modern Biology*. Even the sub-title which states that it is "A review of the principal phenomena of animal life in relation to modern concepts and theories" suggests a far more inclusive scope than the book possesses. The contents of the volume may be indicated briefly. Following an explanatory introduction, Chapter I is devoted to a consideration of mechanistic biology and neo-vitalism, and is largely an adverse criticism of Needham's essay in *Science, Religion and Reality*. The second chapter deals with metabolism, adversely criticising Johnstone's views on the nature of life in relation to entropy expounded in his volume *The Mechanism of Life*. The next four chapters are concerned with evolution and heredity and the part played by mutation, hormones, functional activity and external conditions. The view-point is that of an avowed Lamarckian, the author of a book on *Hormones and Heridity*. These chapters contain accounts of Heslop Harrison's work on induced melanism in Lepidoptera, Guyer's experiments on induced eye defects in rabbits, the work of Tornier and Berndt on abnormalities in goldfish, Kammerer's experiments on Alytes, Salamandra and Proteus and Weldon's twenty-five year old researches on Carcinus. In the final chapter mind and consciousness are discussed.

The book is written in a stimulating but somewhat controversial and forthright manner. It is a little parochial in outlook and the general perspective of values indicates perhaps a slightly myopic vision as is illustrated by the too frequent mention of a particular famous surname when others associated with equally brilliant and more massive work remain unmentioned and the researches unnoticed. It is also written perhaps somewhat carelessly, as is evidenced by the confusion in Chapter I and Index of a clever son with a wise father. Still, Mr Cunningham says many things that have needed saying for some time—that biochemistry and statistics are not biology, and that the purely biological approach to the study of life, of form and structure, of growth, development, heredity and evolution cannot, so far as we can see at present, be replaced by the chemical or statistical approach. Concerning the latter the author writes: "There is a tendency in modern biology to suppose that when structures and functions have been measured and the results expressed in curves,

graphs, or mathematical formulae, the causes of them have been discovered." This might have been expressed more strongly, for the fashionable idolatry of statistics by many biologists may, unless controlled, become a dangerous tendency, doing more to confuse and distort issues than to clarify them.

In his last chapter, on mind and consciousness, Mr Cunningham is writing on a subject which is not entirely his own. Much of the chapter concerns McDougall's "Lamarckian" experiments on rats which are quoted with approval, whereas Hazlitt's destructive criticism of them published in the same journal a few months later is not referred to. In concluding, the author discourses at large of social tendencies and to many of us his views, although interesting, might be regarded as lacking in insight.

As a whole the volume is essentially a statement of Mr Cunningham's well-known position, that evolution and its cognate issues cannot be explained in terms of Mendelism, mutation and natural selection, but that the influence of the environment, of functional activity and of the internal secretions must be taken into consideration as primary factors.

The volume is interesting and very readable, but it is possible to regard it as special pleading in that it is written from a particular point of view. The latter, however, needs expression and demands more respect than it commonly receives. On the whole the book is perhaps just a little disappointing, for it is so good that one feels it might easily have been just a little bit better.

WILLIAM B. BRIERLEY.

## PROCEEDINGS OF THE ASSOCIATION OF ECONOMIC BIOLOGISTS

ORDINARY MEETING held at 2.30 p.m. on Friday, October 26th, 1928, in the Botanical Lecture Theatre of the Imperial College of Science and Technology, London. The Chair was taken by Dr J. WATERSTON, Vice-President.

The following papers were read:

- I. "On the Biology of *Sirex cyaneus* Fabr. (Hymenoptera-Siricidae) and its parasites in Britain" by Mr R. N. CHRYSTAL, M.A., B.Sc. (Imperial Forestry Institute) and Mr J. G. MYERS, Sc.D., F.E.S. (Imperial Bureau of Entomology).
- II. "Notes on a Fungus associated with *Sirex cyaneus*" by Mr K. St G. CARTWRIGHT, B.A. (Imperial College of Science and Technology).

Mr CHRYSTAL gave an account of some biological studies which have been in progress during the past two years on the Sirex woodborer *S. cyaneus* Fabr., and its two parasites, *Rhyssa persuasoria* L. (Hymenoptera-Ichneumonidae) and *Ibalia leucospoides* Hochenw. (Hymenoptera-Cynipidae).

The biology of the woodwasp has been chiefly studied in relation to its attacks on larch, which is probably its principal host tree. The main details of this are now almost completely known, and one of the chief points which the research has brought out is that this insect is a secondary pest from the standpoint of the living tree, and therefore rather an indicator of pathological conditions than the direct cause of them. It has been shown, for example, that trees may be fungus-attacked (*Armillaria*, *Fomes*, etc.) or suffer from the effects of bad soil conditions and yet remain unattacked by the woodwasp for some time. The biology of the parasites, especially the Ichneumonid *R. persuasoria* L., was studied with the primary object of ascertaining whether or not one or both species could be exported to New Zealand as an enemy of a closely allied species of woodwasp (*S. juvencus* L.) which has become established there.

In the work on *Rhyssa* a considerable amount of new data has been accumulated, especially concerning the egg-laying habits, a full account of which was presented for the first time.

The habits of the Cynipid parasite, *Ibalia leucospoides* Hochenw. were, previous to this work, almost completely unknown. A fairly complete record of its biology has been obtained during the past 2 years, the main features of which are as follows.

The parasite has been shown to have an extremely specialised egg-laying habit. The larval stages are now known to be markedly hypermetamorphic and a study of the morphology of these will prove of extreme value in deciding the systematic affinities of the sub-family *Ibaliinae*. Lastly, it has been shown that this parasite, hitherto considered a very rare species in Britain, is to be found prevalent in nearly all localities where *S. cyaneus* is of common occurrence.

The paper was illustrated by lantern slides.

## 182 *Proceedings of the Association of Economic Biologists*

Mr CARTWRIGHT said: Work on the association of insects and fungi has been confined, up to the present almost entirely, to the entomological side. Many of the details in regard to wood-inhabiting insects have been excellently summarised by Paul Buchner (1) in a recent publication entitled *Holznahrung und Symbiose*, which contains drawings showing the presence of symbiotic organisms. A paper of a more exhaustive character by Breitsprecher (2) has just come out. This is mainly a description of sections cut through the abdomens of species of *Anobiidae*, showing the presence of symbionts.

The classical example of an association between insects and fungi is that of the *Ambrosia* beetles. In this case the fungus is confined to a narrow zone round the galleries of the insect and is definitely transmitted by the ovipositing female; the fungus spores being excreted on to the egg coat. A simpler association was described by Möller in his studies of leaf-cutting ants which cultivate fungi for their food supply, here the fungus, *Rozites gongylophora*, is definitely carried by the queen in a special cavity, to the new colony. Other related ants cultivate a fungus on chewed-up wood.

Much indirect evidence about the subject has been obtained from the practical foresters, architects and others who have so frequently noted that insect and fungus attack appear to be connected, as to suggest that some direct association existed between them. In these notes attention will be confined to the association between wood-feeding insects and fungi. This may be considered under two headings: I. Association of an insect with an internal symbiont, *i.e.* the regular presence in the body of the insect of a foreign organism which is often confined to special glands. II. The regular occurrence of a fungus in the wood or tissues of the host in which the insect is living.

I. It is not intended to discuss at any length the question of internal symbionts, as most of the information so far gained has been on the entomological side. Suffice it to say that in most timber beetles and bark beetles which have been examined, bacteria, yeasts or fungi proper have been found to occur; as an example of these, *Endomyces* may be mentioned.

It has been suggested that symbionts will be found to be present in insects which feed on materials such as wood, which are not readily digestible, whilst these will not occur when the insect lives on substances that are easily absorbed. The conclusion drawn is that these symbionts enable the insects to break up the wood, etc., and make available for their assimilation these foodstuffs. It has been assumed that the insect alone is unable to do this. Chemists who have analysed beetle frass have stated that it appears to be unaltered wood. These results, however, need confirmation as it is difficult to suppose that neither the fungus nor the beetle have in any way altered the wood during its passage through the body of the latter. Such would mean that their relationship was in the nature of a mutual parasitism without any outside source of energy. It would seem probable that such alterations as occur are not detectable by the present methods of wood analysis.

The organisms usually occur either in some part of the gut, into the epithelial layer of which they may work their way, or they may be contained in special glands opening into the vagina, being transmitted to the egg coating either before or during oviposition. The larva in hatching from the egg eats part at least of the egg coating and thus becomes infected.

Unfortunately up to the present no proof, as far as I am aware, has been brought forward that the internal symbionts do really help in the breaking up of the wood and all attempts to obtain these insects free from the organisms have so far failed. Until the technique for achieving this has been worked out, it is by no means certain that the insect really is dependent on its internal symbiont. Should it be found that the insect can live independently of any symbiont it does not rule out the possibility that such insects may not require wood already altered by fungus action or that in some instances the insect larva may be entirely mycophagous.

Although the sterilisation of the insect appears to be necessary to furnish conclusive evidence one way or the other, another line of attack which would afford a certain amount of circumstantial evidence is that of isolating these symbionts from the insects and growing them on artificial media. The actual technique of isolation may prove difficult, but should not be insurmountable. If this could be done, it could at any rate be ascertained whether they were capable of breaking up any of the wood constituents. Up to the present material has not been available on which to attempt this work, but it is hoped to undertake it in the near future.

## II. ASSOCIATION OF INSECTS WITH FUNGI LIVING EXTERNALLY IN THE SURROUNDING MATERIAL.

From the examination, by means of sectioning, of much wood attacked by beetle, it has been ascertained that wherever larval tunnels are present, there invariably can fungus mycelium be found. Up to the present no case has come under my observation in which this did not prove to be true. In the case of bark beetles for example, the tunnels are often seen lined with fungus which in certain instances is a species of *Endomyces*. Schneider-Orellis found thick-walled fungus spores in the gut of the overwintering female; these germinated readily in the excrement of the insects. During the examination of some ash wood attacked by *Daldinia concentrica*, tunnels of *Hylesinus fraxini* were observed; these were seen to follow closely the black zone lines caused by the fungus. In old beams and furniture there would appear also to exist some connection between beetle attack and the presence of fungi. Furniture which had been in store for about 8 years was found to be attacked by beetle and an examination showed that fungus mycelium was present.

The moisture content in old furniture, beams, etc., may be as low as 7 per cent. and is rarely above 15 per cent. Such low moisture contents would appear to exclude the possibility of finding mycelium in a state of active growth.

The fungi obtained by cultural methods from such old wood, which is often very much broken up by larval galleries, cannot be assumed to be those primarily concerned. It is highly probable that in many cases the fungi perform their work prior to the insect attack and that the beetle larvae feed either on the altered wood or on the dead fungus mycelium or on both.

Many different fungi commonly present in wood, such as *Trichoderma lignorum*, *Torula* sp., *Penicillium* spp., etc., have appeared in such cultures taken from near beetle tunnels. So far my work on the association of timber-destroying beetles and fungi has been confined to the examination of beetle-attacked wood for the presence of fungus and to keeping in culture those fungi which are isolated frequently from cultures taken near the tunnels or from frass.

Only in two instances has a Basidiomycete been obtained. *Salix* wood, containing tunnels of *Xestobium*, was decayed throughout by *Fomes applanatus*, but the beetle attack had obviously come in later and there was no question of the fungus having been introduced by the insect. A *Xestobium* larva has since been placed on a pure culture of this fungus growing on malt agar and it has been alive for about 1 month in the culture, on which it feeds to a certain extent. The second case was also on *Salix*, from which, amongst other fungi, a member of the Cyphellaceae has been obtained. In several beetle-attacked oak samples sent for examination, a fungus with conidia of the *Septocylindrium* type has been found and a species of *Torula* was also present.

These fungi have often been observed in old oak which shows "golden" coloration; from this *Eidamia catenulata* has also been isolated on several occasions. It would be interesting to ascertain whether these richly coloured and much sought after specimens of old oak are more susceptible to insect attack than is the normal, as Prof. Groom (3) and Mrs Williamson (4) have shown that such coloration can be caused by fungus action.

Finally it may be mentioned that a fungus having the  $\beta$  type spore of a *Phomopsis* has been isolated by Mr Day and Mr Nutman from *Lyctus*-attacked wood. This has not yet been identified with certainty and may possibly prove to be a species of *Cytospora*.

To summarise, two explanations of the regular presence of fungus in and around beetle tunnels may be put forward: (1) the fungus already present grows more actively in the region of the beetle tunnels because the aeration is better there and because it derives nourishment from the waste products of the insect which may tend to raise the moisture content of the wood around the tunnel; or (2) the insects make their galleries in that part of the wood where there is most fungus mycelium and also they spread the fungus by infection carried on their bodies.

Probably both explanations hold good to some degree and the fungus once introduced finds the conditions surrounding the tunnel the most favourable for its growth.

We may conclude, tentatively, that beetles always prefer wood which contains fungus, because (1) they are better able to digest wood which has been attacked by the enzymes of a fungus, and (2) they derive part or all of their food supply from the actual mycelium of the fungus, dead or alive. It is not to be expected that any wood-destroying fungi will be found living and growing actively in wood with a moisture content as low as that usually found in old oak beams, etc., as they require in most cases a moisture content about that of fibre saturation point (28 to 30 per cent.). It would be interesting to ascertain the lowest moisture content at which the various fungi isolated from beetle-infested wood could grow, and also whether the moisture content of the wood in the neighbourhood of the galleries was increased by the metabolism of the insect.

#### *Sirex cyaneus and fungus association.*

The study, of which this is an account, was undertaken on the suggestion of Mr Chrystal after a meeting of this Society on March 23rd of this year, and the work is as yet quite incomplete; thus only tentative conclusions can be drawn as to the results.

Attention was drawn to the paper of Buchner in which it is stated that oidia of a fungus had been found in a gland or squirt at the base of the ovipositor in *Sirex*

*gigas*. These glands Buchner figures. They are paired and open out into the vagina, the oidia being extruded on to the eggs after they have left the ovaries. Furthermore, he had observed clamp connections, proving that the fungus belonged to the Basidiomycetes, though in his figures these are by no means convincing, as they are in no case complete, the septa being absent. Certainly one would suspect them of belonging to a Basidiomycete.

Slides prepared at Oxford were sent me for examination in April. These showed oviposition and young larval tunnels, and in every case a mass of mycelium was present in the wood around the vessels. The outer sculptured wall of an ovum was left *in situ* in one of the tunnels and showed hyphae around it. At the same time slips of larch wood with oviposition tunnels and young larval galleries were sent, which, on sectioning, showed mycelium of a similar kind with clamp connections conspicuously present. From an examination of further specimens, both of wood attacked by *S. cyaneus* and of that attacked by *S. gigas*, it would appear that a fungus mycelium belonging to a Basidiomycete is always present in the wood in which these larvae are living. Furthermore, this mycelium in every case seemed to have its focus around the galleries and was definitely attacking the wood, which showed numerous bore-holes. The moisture contents of one sample were determined and found to range between 35 per cent. and 48 per cent., a condition of the wood which would be favourable for the growth of these wood fungi. From the original samples, culture plugs were taken and a fungus which grew readily on a 2 per cent. malt or prune agar, was isolated. This mycelium soon produced numerous clamp connections and was moderately rapid in growth. The culture remained white for some months, but now colour is developing in some of them. They are of a soft downy texture. The same fungus was isolated from several different samples. So far the cultures have shown no sign of fruiting though undifferentiated segments of hyphae separate off and may act as oidia.

The small specimens from which the cultures were taken already showed fungus mycelium throughout, though it was more plentiful in the neighbourhood of the tunnels. One could not feel certain, therefore, that this wood had not contained fungus before the *Sirex* attack. However, in August, material was obtained which contained oviposition tunnels and eggs of about 2 weeks old. These were sectioned and in every case the same type of mycelium was seen having the tunnel as its focus of development, the mycelium rapidly diminishing in amount away from the tunnel. Sections from areas closely adjacent to these tunnels showed no trace of fungus; demonstrating clearly that this really had been introduced by *Sirex* during oviposition.

The extent of development of the fungus after 2 weeks was sufficient to suggest that the 5 weeks' interval between oviposition and the hatching would be enough to allow the fungus to make sufficient growth to assist in the nutrition of the larva.

A few dead female adults and a number of half-grown larvae were obtained in August, the majority of these were fixed and have been embedded for the cutting of serial sections. One of the females was dissected and the eggs removed from the uterus and examined under the microscope. A fine mantle of mycelium was seen in between, and surrounding the eggs closely. No clamp connections were seen in this mycelium.

A few of the eggs were placed in slants of 2 per cent. malt agar: these cultures became contaminated but sufficient could be seen in the mixed culture to show the

presence in one case of a Basidiomycete. Eggs were also extracted from oviposition tunnels made about 2 weeks previously; of these, ten were placed on 2 per cent. prune agar slants and six on malt agar one egg being placed in each tube. Out of the prune tubes three gave cultures of mycelium showing the same characteristics as that of the fungus originally isolated from the wood, and bore clamp connections; four remained sterile, and two gave contaminants. Out of the malt, two gave mycelium with clamps and three remained sterile. From the tenth prune tube and the sixth malt tube the eggs were removed and found still to show the mantle of mycelium as seen round the eggs dissected from a female *Sirex*<sup>1</sup>. No fungus has been found around the eggs in the ovaries; further dissections will have to be made to settle this point definitely. A cursory examination of a few larvae showed mycelium to be present in a disorganised condition as if partly digested, but mycelium which appeared undisorganised was also seen. It is thought probable, therefore, that this may remain in a resting condition through the pupal stage and be found in the adult as described by Buchner.

A newly hatched larva, removed from its tunnel, was placed on a culture of the fungus where it lived for 3 weeks apparently feeding on the fungus. One of the half-grown larvae lived for 3 months on cultures of this fungus. It was transferred from time to time to fresh cultures which it could be definitely seen to eat.

#### *Identification of the fungus.*

The culture of the fungus from *Sirex cyaneus* agrees with none of the fairly numerous type cultures which I keep for comparative purposes. No clue has so far been vouchsafed either in the finding of sporophores on the trees or in the type of rot produced. It is probable that identification will be secured when more comparative cultures are obtained.

In regard to *Sirex gigas*, the species with which Buchner chiefly deals, I have only had a few specimens from some larch props sent for another purpose.

From an examination of these samples which contained pupae and from which adults hatched out in the laboratory, it would appear that a similar association exists here also.

#### CONCLUSIONS.

To sum up, it appears that sufficient evidence of a circumstantial nature has been brought forward to show that:

1. A Basidiomycete fungus is always present in wood-containing *Sirex cyaneus*.
2. This fungus has proved so far to be identical in every case examined.
3. That the fungus is introduced with the egg during oviposition. In the cases examined this has been in the form of a fine mycelium which, being in the primary condition at the time of oviposition, has no clamp connections. It develops these, however, both in the wood and in artificial media.
4. The fungus causes a rot of the wood in which bore-holes are made.
5. It can advance sufficiently in the time elapsing between oviposition and the hatching out of the young larva either to have formed food for the larva itself or to have acted upon the wood to a sufficient extent to make this available as food.

<sup>1</sup> Some of the cultures which had remained sterile are now showing fungus development.



6. The larva can live and definitely grow on a pure culture of the fungus for a period of at least 3 months, showing that it can derive some nourishment at any rate from eating fungus alone. Further information is needed about the methods whereby the fungus is transmitted from the larval through the pupal stage to the adult, and at what stage in the life-history of the insect the special glands containing the fungus are formed.

Probably some stimulus due either to fertilisation or gland secretion, when the resting oidia are extruded from the gland into the vagina, starts growth.

In the case of *Sirex gigas* the fungus may also be introduced into the wood as oidia: details of this have still to be worked out. It is hoped next season to obtain cultures from eggs directly after oviposition and possibly even to induce egg-laying directly into a medium. Material was not available for this during last summer.

Finally, I wish to acknowledge the debt I owe to the previous speaker who started me on this work and who, with the skillful help of his assistant, has provided me with such excellent material.

#### REFERENCES.

- (1) BUCHNER, PAUL. (1928). *Holznahrung und Symbiose*. Berlin: Julius Springer.
- (2) BREITSPRECHER, E. (1928). Beiträge zur Kenntnis der Anobiiden symbiose. *Zeitschrift für Morphologie und Ökologie*, Band II, Heft 34, August.
- (3) GROOM, PERCY (1915). "Brown Oak" and its Origin. *Ann. Bot.* xxix, No. 115, July.
- (4) WILLIAMSON, HELEN S. (1923). The Origin of "Golden Oak." *Ann. Bot.* xxxvii, No. 147, July.

The following letter has been received by the Editors of the *Annals of Applied Biology*:

ABOL RESEARCH LABORATORIES,  
PADDOCK WOOD,  
KENT.  
October 1928.

DEAR SIRs,

With reference to the article on "The use of Tetrachlorethane for Commercial Glasshouse Fumigation," which was published under my name in the May issue of your *Journal*, I regret that I failed to acknowledge that I was indebted for a considerable part of my information to Messrs Murphy and Son, Ltd., as a result of investigations which I made for them while engaged by them. I omitted to mention in the article that the introduction of tetrachlorethane into Commercial Horticultural practice by the writer in 1920 was in fact effected through Messrs Murphy and Son, Ltd., and was as a result of the investigations referred to above.

Yours faithfully,  
(Signed) THEODORE PARKER.

THE EDITORS,  
*The Annals of Applied Biology*,  
Cambridge University Press,  
Fetter Lane, E.C. 4.

ORDINARY MEETING of the Association held at 2.30 p.m. on November 23rd, in the Imperial College of Science and Technology, South Kensington. The President, Dr E. J. BUTLER, C.I.E., F.R.S., in the Chair.

- I. "The Relation of Environmental Conditions to Angular Leaf Spot Disease of Cotton (*Bacterium malvacearum*)" by Mr R. H. STOUGHTON, B.Sc. (Department of Mycology, Rothamsted Experimental Station, Harpenden, Herts.).
- II. "The Effect of Environmental Conditions on Plant Diseases under Glass" by Dr W. F. BEWLEY (Director, Experimental and Research Station, Cheshunt, Herts.).
- III. "Temperature and Humidity in Relation to Tomato Mildew (*Cladosporium fulvum*)" by Mr T. SMALL, B.Sc. (Experimental and Research Station, Cheshunt, Herts.).

# I. THE RELATION OF ENVIRONMENTAL CONDITIONS TO ANGULAR LEAF SPOT DISEASE OF COTTON (*BACTERIUM MALVACEARUM* E.F.S.).

BY R. H. STOUGHTON, B.Sc., A.R.C.Sc.

THAT the environment plays a large part in conditioning the incidence and development of plant diseases has long been recognised. The practical difficulties inherent in the study of the effect of external factors on an exact basis, and the comparatively greater ease with which the behaviour of the causal organism in pure culture may be investigated, have, however, led plant pathologists until the last decade or so to focus their attention mainly on this latter aspect. Such information as we have on the influence of environmental conditions has largely been based on observational rather than exact experimental data. Apart, also, from the practical difficulties of the study of these factors under known controlled conditions, the problem is further complicated by the fact that the environment influences not only the parasite but also the host plant. The disease, *qua* disease, is the resultant of the activities of the host and of the parasite, and valuable information on this resultant can only be obtained from a knowledge of the two parts that go to make it up.

Yet another difficulty and one that requires intensive investigation, is the obtaining of some precise quantitative measure of "degree of attack." A mere statement of numerical percentage of diseased plants or seedlings under any given conditions, a criterion which is often taken as a measure of degree of incidence, seems to give only half the truth, expressing only the degree of absolute resistance within the limited population of plants studied. A measure is needed which will give some value to the *severity* of attack in each individual case, in terms of some physical dimension such as length or volume of diseased tissue.

## *Proceedings of the Association of Economic Biologists* 189

Turning to the practical aspects of control of environmental conditions the factors that may be concerned can be tabulated as follows:

Physical	Chemical	Biological
Soil Temperature	Soil Nutrients	Flora and Fauna of
„ Moisture	Air, CO <sub>2</sub> content	the Soil
„ Aeration		
„ Reaction		
„ Condition		
Air Temperature		
„ Humidity		
„ Movement		
Light Intensity		
„ Quality		

In the study at Rothamsted of the effect of the environment in conditioning the incidence and development of the angular leaf spot disease of cotton attempts have been made to control these factors, starting with the simplest apparatus and gradually working up to apparatus in which as many factors as possible can be controlled.

The first investigation was concerned only with soil temperature in an attempt to confirm or disprove Massey's theory of the influence of this factor on primary infections of seedlings from infected seed. The work was carried out in a series of differential temperature tanks on the principle of the Ganong incubator, one end of the series being maintained at a constant high temperature (37° C.) and the other at a low temperature (13° C.), the intermediate tanks showing a range of temperatures between these extremes. Results were so conflicting that it was felt that the effect of soil temperature could not be differentiated from that of other factors, and the next step was the construction of an infection-chamber in which the entire plant could be placed under conditions of known air (and, of course, soil) temperature and humidity. With this apparatus the problem was attacked from the point of view of secondary infection, seedlings raised in the glasshouse being sprayed with a strong suspension of the organism, placed in the chamber for 48 hours, removed to the glasshouse and watched for the development of the disease. Briefly, it was found with this type of apparatus that the limiting temperature for infection with the organism was 32° C. Below this temperature the incidence was governed by the prevailing humidity. At a temperature of 28° C. infection took place with a humidity of over 70 per cent. relative saturation, but at lower humidities infection did not occur. On reducing the temperature to 25° C., however, infection could take place even at a relative humidity of 65 per cent. It is seen, therefore, that humidity and temperature are interrelated factors, the limiting humidity for infection depending on the temperature and *vice versa*.

These results were of sufficient importance for the Empire Marketing Board to take an interest in the investigation, and a grant was made for the construction of more elaborate pieces of apparatus in which it is hoped that all the factors tabulated previously may be controlled to an exact degree. They consist essentially of modified Wisconsin tanks provided with eight soil containers, and having above them glass-walled air chambers with powerful electric lights suspended close to the top. A film of water is kept flowing over the top to prevent the heat from the lamps entering the

chamber. Air temperature is controlled within a range of  $1\frac{1}{2}^{\circ}$  to  $2^{\circ}$  C. by means of a sensitive thermostat incorporating a bimetallic strip, the heat being provided by two carbon filament lamps at the sides of the chamber. Humidity is kept constant by a device depending on the vaporisation of water from wet muslin covering a resistance lamp enclosed in a tin through which an air stream is blown, the lamp being controlled through a relay by a hair hygrostat within the chamber. The air within the chamber is kept in circulation by a fan, and the air stream from the humidifier ensures constant changing of the atmosphere. Six of these chambers are being constructed and should be in working order early in 1929.

The paper was illustrated by lantern slides, showing the details of the several pieces of apparatus described and giving tabulated results obtained in experiments.

## II. THE EFFECT OF ENVIRONMENTAL CONDITIONS ON PLANT DISEASES UNDER GLASS.

BY W. F. BEWLEY, D.Sc.

OBSERVANT nurserymen have long realised in a broad way that the diseases attacking their plants are most serious under certain conditions of temperature and humidity, and some sort of control has been obtained by avoiding these conditions as much as possible. Observations such as these have been based largely upon the "feel" of the atmosphere on entering a house, or upon records taken with the aid of cheap and somewhat crude instruments. It is obvious that more careful observations made with accurate instruments, supplemented by experiments under properly controlled conditions must lead to vastly improved methods of control. The glasshouse industry teems with problems of this kind, and while most diseases are under some measure of cultural control, there is need for more concentrated effort before we can be reasonably satisfied with our methods.

It is only of recent years that an attempt has been made to study at all carefully the effect of physical factors upon plant diseases, and much of the pioneer work on temperature is due to the United States Department of Agriculture and Prof. L. R. Jones and his colleagues at the University of Wisconsin. This work, and subsequent papers, can be read by all who wish, and there is no need to discuss it here. It is common knowledge that the physical factors of the environment affect (1) the organism, (2) the host plant, and (3) the disease complex. The grower, whom we are trying to help, is mainly concerned with the second and third.

The effect of temperature can be seen early in the case of the tomato, especially in the first 2 months after planting. Seeds sown in December are held at a temperature of  $16^{\circ}$  C. Soil temperature at planting time is important; it should be  $16^{\circ}$  C. and certainly not lower than  $14^{\circ}$  C. At this temperature the roots continue their development in the new soil, and growth of the plant proceeds steadily. At soil temperatures below  $14^{\circ}$  C. root development is checked, some roots die, the leaves become purplish green and the growth rate is definitely retarded.

Air temperature after planting has a marked effect on the plant, as was seen during the past season in chambers where minimum night temperatures of  $13^{\circ}$  C.,  $16^{\circ}$  C.,  $17^{\circ}$  C.,  $18^{\circ}$  C. and  $21^{\circ}$  C. were maintained by thermostatic control.

An excellent type of growth was obtained at  $17^{\circ}$  C. and  $18^{\circ}$  C. The fruit set freely and the total weight was 28 per cent. greater than that at  $16^{\circ}$  C. and  $21^{\circ}$  C. Growth at  $16^{\circ}$  C. was sturdier, darker in colour and slower, while at  $13^{\circ}$  C. the

plants were much retarded and purplish green in colour. At 21° C. growth was too rapid and soft. The plants were etiolated and the two bottom trusses failed to grow more than 1 in. in length. The plants developed "Stripe" disease very badly.

The amount of sunlight must be considered in relation to temperature. Bright sunlight matures the tissues and hardens the growth and therefore counteracts the softening effect of high temperatures. The successful cultivator of glasshouse-plants reduces the temperature in dull weather and drives his boilers in sunny weather. Sudden changes in temperature are important, especially differences between day and night temperature. This was especially noticeable this season, where, in spite of the sunny days, the night temperature has been abnormally low. The worst effect was seen in the spring when a day temperature of 41° C. followed by a 13° C. night temperature was common. As a result, the pollen did not disperse freely and the bottom trusses set badly.

#### THE EFFECT OF ENVIRONMENTAL CONDITIONS UPON CERTAIN DISEASES.

##### 1. *Tomato diseases.*

*Damping-off and foot rot of the tomato.* *Phytophthora parasitica* is most dangerous during the June sowings. Many cases are recorded where seed sown in soil taken from the same heap is practically free from damping-off in December, and shows high mortality in June. This is explained by the optimum temperature of about 27° C. *Phytophthora cryptogea* has a slightly lower optimum for infection and spread, namely 24° C. *Rhizoctonia solani* has a still lower optimum at about 18° C. The damage done by all three is comparatively small below 10° C. All three require high humidities for maximum effect. Drying the surface of the box by mechanical raking helps to control "damping-off" due to *Phytophthora* but not *Rhizoctonia*.

*Thielavia basicola* attacks tomatoes in the pot stage and rarely continues after planting out. Infection experiments have given best results at 13° C., but temperature is not the only factor. Root infection by this fungus depends upon the growth rate: the disease spreading in the tissues most rapidly when the plant is growing slowly. Almost any method of increasing the rate of growth of the plant, will check the development of this disease.

The appearance of *Stripe disease* is governed by the state of the plant, those showing soft and rapid growth being more susceptible than more slowly growing harder plants. Abundant sunshine matures the tissues, hardens the growth and checks outbreaks of this disease. Dull weather and high humidities cause severe outbreaks. Temperatures too are important, for above an average day temperature of 24° C. Stripe fails to develop rapidly.

*Sleepy disease* caused by *Verticillium albo-atrum* is typically one of low temperatures and its appearance can be forecasted with uncanny accuracy by considering the temperatures during the first fortnight after planting. Temperatures between 16° C. and 24° C., with an optimum around 21° C. to 23° C., are favourable to the rapid progress of this wilt. Plants wilt at temperatures around 16° C. and recover when transferred to temperatures above 25° C. This is no doubt due to the fact that toxins capable of producing wilt are not produced by the fungus at high temperatures.

A method of cultural control applicable when a large proportion of the crop is attacked has been satisfactorily employed as follows: (1) overhead damping in place

## 192 *Proceedings of the Association of Economic Biologists*

of watering; (2) shading the houses; (3) maintaining an average temperature about 25° C.

*Fusarium lycopersici* rarely occurs in this country, although root rot caused by *F. oxysporum*, etc., is relatively common. Apparently the soil temperature is too low for this fungus.

### 2. *Cucumber diseases.*

*Colletotrichum oligochaetum*, which causes the dreaded "spot" disease of cucumbers, has been eradicated by house hygiene including steam sterilisation of the soil and disinfection of the superstructure with cresylic acid or formaldehyde. At the height of its activity in 1920–1923 it was held in partial check by controlling the temperature.

Investigation of the disease in the Lea Valley indicated its temperature relations as a minimum temperature of 7° C., a maximum of 30° C., with the optimum around 24° C. The rate of progress of the disease was materially checked by maintaining an average temperature above 30° C. Obviously high humidities favoured the disease.

The temperature and humidity relations of "gummosis" of the cucumber due to *Cladosporium cucumerinum* have not been determined, but it is known that cool, moist conditions favour its appearance. It occurs mainly during cold Springs and Autumns, and rarely during normal cucumber conditions.

The control of *Mosaic disease* is much too complicated to be achieved by environmental conditions only, but certain observations with cucumber mosaic are important commercially. The symptoms appear on leaves, flowers and fruits, but usually the fruits are not affected at temperatures below approximately 27° C. In nurseries fruit damage is obviated by suitable ventilation, which prevents the house atmosphere from rising above 27° C.

## III. TEMPERATURE AND HUMIDITY IN RELATION TO TOMATO MILDEW (*CLADOSPORIUM FULVUM*).

By Mr T. SMALL, B.Sc.

In temperate countries tomato mildew (*Cladosporium fulvum*) is almost restricted to plants under glass, which suggests that a close relation exists between the disease and environmental conditions. Temperature and humidity are the chief factors concerned. Experiments made under controlled conditions showed that the optimum temperature for *Cladosporium fulvum* is about 22° C. Below this temperature the progress of the disease is retarded, but retardation is not appreciable until temperatures about 15° C. are reached. Such low temperatures merely check the development of the disease subsequent to infection, for severe infections take place under humid conditions at 13° C.

A study of the effect of humidity on tomato mildew showed that *C. fulvum*, in common with most fungi, is favoured by very humid conditions. At 22° C., infection is severe at 80 per cent. humidity but is rare at 70 per cent. humidity. The fungus sporulated abundantly on diseased plants exposed to 80 per cent. humidity at 20° C. but produced very few spores at 58 per cent. humidity.

Figures were quoted, calculated from continuous records, to convey some idea of the very high night humidities existing in commercial glasshouses. Experimental evidence was given to support the conclusion that the presence and persistence of such high humidities is mainly, and in some cases solely, responsible for tomato mildew.

ORDINARY MEETING held at 2.30 p.m. on Friday, December 14th, in the Imperial College of Science. The President, Dr E. J. BUTLER, C.I.E., F.R.S., in the Chair.

### THE ANTISEPTIC PRESERVATION OF WOOD<sup>1</sup>.

By Professor PERCY GROOM, F.R.S.

THE paper deals with felled timber and almost exclusively with preservation against fungi and thus largely excludes from consideration attacks by insects on wood.

Moreover, in all investigated cases of attack on wood by insects these cannot feed on wood without the co-operation of symbiotic protozoa, bacteria, or fungi. A number of chemical elements, especially arsenic, fluorine and mercury, provide potent preservatives against both insects and fungi attacking wood.

National economic loss is caused by the decay of wood mainly due to neglect of sanitation (*e.g.* houses) and of application of preservatives (*e.g.* mines), or by inappropriate preservative treatment (*e.g.* creosoted paving). Appropriate preservative methods can be adopted to increase the utilisation of rapidly-grown perishable timbers in temperate and tropical countries.

A sharp distinction must be drawn between mere temporary antiseptics and prolonged preservation. The duration of the protection afforded by fungicides depends upon the depth of penetration and the nature of the fungicide. Mere superficial treatment of the wood provides the shortest protection, as untreated internal wood is liable to be exposed by drying, abrasion and so forth, and the fungicide is liable to diminution by washing away, evaporation and chemical change.

#### *Methods of application.*

Preservatives may be applied to the whole of the piece of timber or solely to the part that is most vulnerable to attack: the former may be called *general* and the latter *regional* treatment.

*Superficial coatings.* The most ancient superficial coat is represented by the bark, and dating back to prehistoric times is the *charring* of wood by flames. *Antiseptic liquids* are applied either to prevent the germination of spores or to provide prolonged protection. Many weakly antiseptic solutions, such as sodium carbonate, serve to provide transitory protection against the germination of spores. For more prolonged preservation aqueous solutions may be used where the wood is not exposed to rain and may be applied hot or cold; but where liable to be washed away the liquid must be a tar-oil.

*Excavation and slits.* Even in ancient times holes were driven into the wood and preservatives were deposited in these with the object of increasing the depth of penetration. This method is now mainly used in the regional treatment of posts, the auger-holes being made just above and just below ground level.

Allied to this method is the very novel *Cobra* method, which may be described as one of *inoculation*. This is used to protect telegraph poles by treatment of freshly-

<sup>1</sup> As this Paper was written for and will be published in the *Empire Forestry Journal*, only a brief abstract of it is given here.

felled poles in the region that will be near the ground level. The preservative takes the form of a concentrated fungicidal paste (fluoride and 2 : 4 dinitrophenate of sodium) which is injected into the wood by means of a very large, flat, hollow inoculating needle. The "needle" merely separates the fibres, producing slits. The slits are distributed round the post for a length of a metre, but owing to the diffusion of the ingredients by the water originally in the wood the preservatives eventually form a closed protective belt round the whole of the sapwood for a length of two metres. Similar but much less extensive treatment is adopted at the top of the telegraph pole where the electric wires are fixed, and finally the pole is painted with a coat of a tar-oil preservative. The Cobra apparatus is portable.

The so-called *Boucherie* methods of impregnating wood by suction or hydrostatic pressure were considered in the paper together with modern variants of these.

*Submersion.* The depth of penetration ensured by submerging wood in a preservative solution varies greatly with the time of submersion, often the temperature of the solution, and the nature and condition of the wood. The wood to be treated must be seasoned. The treatment may be general or regional.

*Pneumatic pressure.* This was illustrated by a brief description of the ordinary method of creosoting timber and by reference to methods of economy by means of partial recovery of the creosote, or by means of an admixture of cheaper liquids.

#### *Antiseptics.*

In the past the fungicidal efficiency of liquids designed for protection of wood has been tested by experiments on cultures of various kinds of fungi on gelatine and agar media, and on wood. Misleading results have been obtained because due allowance has not been made for the fact that different species of fungi differ widely in their resistance to the same fungicide, and the fungicidal power of a given liquid on a given species of fungus may differ widely with the nutrient medium employed. Accurate results can be obtained only by testing cultures of wood-attacking fungi feeding on wood, and even in these cultures the effects of temperature and water-content intervene.

As a result of his most recent cultures on wood Prof. Richard Falck ranges fungicides in five groups, beginning with the most powerful as represented by: arsenic (arsenites and arsenates); fluorine (fluorides, silico-fluorides) and corrosive sublimate; dinitrophenol, etc.; heavy metals (copper sulphate and zinc chloride) and tar-oils.

It is evident, however, that the fungicidal efficiency of a substance does not give an accurate indication of the preservative value of this, since the substance may undergo loss by leaching out, evaporation, chemical change; or it may weaken the wood, or be dangerous by reason of its explosive or poisonous properties. Accordingly, the paper included a detailed discussion of a number of timber preservatives.



## LIST OF MEMBERS OF THE ASSOCIATION OF ECONOMIC BIOLOGISTS.

### HONORARY MEMBERS

- APPEL, Geheimer Reg. Rat Prof. Dr OTTO, Biologische Reichsanstalt f. Land- u. Forstwirtschaft, Dahlem, Berlin.
- BELJERINCK, Prof. Dr M. W., Gorsel, near Deventer, Holland.
- CHEVALLIER, Directeur Aug., Laboratoire d'Agronomie coloniale, 57, Rue Cuvier, Paris (V<sup>c</sup>).
- EHLE, Prof. NILSSON, University of Lund, Alliark, Sweden.
- HOPKINS, Dr A. D., Bureau of Entomology, Department of Agriculture, Washington, D.C., U.S.A.
- HOWARD, Dr L. O., Bureau of Entomology, Department of Agriculture, Washington, D.C., U.S.A.
- JONES, Prof. L. R., University of Wisconsin, Madison, U.S.A.
- MARCHAL, Prof. P., Institut National Agronomique, 16, Rue Claude Bernard, Paris.
- NEUMANN, Prof. L. G., École Nationale Vétérinaire, Toulouse, France.
- RAILLIET, Prof. ALFORT, Paris.
- SILVESTRI, Prof. F., R. Scuola Sup. d'Agricoltura di Portici, Naples, Italy.

### ORDINARY MEMBERS (*Life Members are marked \**)

- 1920 ALCOCK, Mrs N. L., F.L.S., Royal Botanic Gardens, Edinburgh.
- 1914 ANSTEAD, R. D., C.I.E., M.A., 412 Triplecane, Madras, S. India.
- 1927 ARCHIBALD, Major R. G., M.D., Director Wellcome Tropical Research Laboratories, Gordon College, Khartoum, Sudan.
- 1924 ARMSTRONG, S. F., 84, Hills Avenue, Cambridge.
- 1908 ASHWORTH, Prof. J. H., D.Sc., F.Z.S., F.R.S., 69, Braid Avenue, Edinburgh.
- 1914 BAILEY, M. A., Botanical Section, Ministry of Agriculture, Khartoum, North Sudan.
- 1926 BAL, D. V., Agricultural Research Institute, Nagpur, India.
- 1926 BALFOUR-BROWNE, Prof. F., M.A., Winscombe Court, Winscombe, Somerset.
- 1919 BALLS, W. LAWRENCE, Sc.D., M.A., F.R.S., Botanical Section, Ministry of Agriculture, Giza, Cairo, Egypt.
- 1914 BARKER, Prof. B. T. P., M.A., National Fruit and Cider Institute, Long Ashton, Bristol.
- 1927 BARNES, H. F., Ph.D., B.A., Rothamsted Experimental Station, Harpenden, Herts.
- 1922 BARRATT, Miss K., D.Sc., Principal, Horticultural College, Swanley, Kent.
- 1929 BARRITT, M.A., 29, Park Road East, Birkenhead, Cheshire.
- 1923 BAXTER, D. EYRE, 14, Cumberland Walk, Tunbridge Wells.
- 1927 BEER, R., "Astley," Magpie Hall Lane, Bickley, Kent.

- 1928 BENNETT, F. F., B.Sc., N.D.A., Agricultural Department, Armstrong College,  
Newcastle-upon-Tyne.
- 1920 BERRIDGE, Miss E. M., D.Sc., F.L.S., Botany School, Imperial College of Science,  
London, S.W. 7.
- 1919 BEWLEY, W. F., D.Sc., Director, Research and Experimental Station, Cheshunt,  
Herts.
- 1915 BIJL, VAN DER, Prof. P. A., M.A., D.Sc., F.L.S., University of Stellenbosch,  
Stellenbosch, Union of S. Africa.
- 1927 BISSETT, N., M.R.C.V.S., University College, Newport Road, Cardiff.
- 1920 BLACKMAN, Prof. F. F., M.A., Sc.D., F.R.S., St John's College, Cambridge.
- 1919 BLACKMAN, Prof. V. H., M.A., Sc.D., F.R.S., Imperial College of Science,  
London, S.W. 7.
- 1927 BOLLAND, B. G. C., M.A., Caisa Postal 130, Ceará, Brazil.
- 1920 BORTHWICK, Prof. A. W., O.B.E., D.Sc., School of Forestry, University of  
Aberdeen.
- 1923 BOYLE, Prof. CONNELL, M.A., Ph.D., D.I.C., University College, Cork.
- 1920 BRADE-BIRKS, Rev. S. GRAHAM, M.Sc., D.Sc., S.E. Agricultural College, Wye,  
Ashford, Kent.
- 1919 BRENCHEY, Miss W., D.Sc., F.L.S., F.E.S., Rothamsted Experimental Station,  
Harpenden, Herts.
- 1914 BRIERLEY, W. B., D.Sc., F.L.S., F.R.A.I., Department of Mycology, Rotham-  
sted Experimental Station, Harpenden, Herts.
- 1926 BRIGHT, T. B., Chucks Cottage, Walton-on-the-Hill, Surrey.
- 1914 BROOKS, F. T., M.A., F.L.S., The Botany School, Cambridge.
- 1921 BROOKS, R. ST-JOHN, M.D., M.A., D.P.H., D.T.M. and H., Lister Institute,  
Chelsea Gardens, London, S.W. 1.
- 1924 BROWN, Prof. W., M.A., D.Sc., Imperial College of Science, South Kensington,  
S.W. 7.
- 1924 BUCKHURST, A. S., A.R.C.S., D.I.C., Pathological Laboratory, Milton Road,  
Harpenden, Herts.
- 1920 BUDDIN, W., M.A., Laboratory of Plant Pathology, University of Reading.
- 1922 BUNYARD, G. N., 25, Bower Mount Road, Maidstone, Kent.
- 1920 BUTLER, E. J., D.Sc., C.I.E., M.B., F.R.S., F.L.S., Director, Imperial Bureau of  
Mycology, Kew, Surrey.
- 1928 CALLAGHAN, A. R., B.Sc. Agr., Department of Agriculture, Sydney, New South  
Wales, Australia.
- Orig. CARPENTER, Prof. G. H., D.Sc., Keeper, Manchester Museum, The University,  
Manchester.
- 1927 CARROLL, J., B. Agr. Sc., 110, Cook Street, Ithaca, New York State, U.S.A.
- 1914 CAYLEY, Miss D. M., John Innes Horticultural Institute, Merton, Surrey,  
S.W. 19.
- 1905 CHANDLER, S. E., D.Sc., F.L.S., Imperial Institute, London, S.W. 7.
- 1925 CHEAL, W. F., Wellington House, Kirton, near Boston, Lincs.
- 1926 CHEESMAN, Prof. E. E., B.Sc., A.R.C.S., Imperial College of Tropical Agri-  
culture, Trinidad.
- 1919 CHIPP, Major T. F., M.C., Ph.D., D.Sc., Assistant Director, Royal Botanic  
Gardens, Kew, Surrey.

- 1908 CHITTENDEN, F. J., F.L.S., V.M.H., Director, R.H.S. Gardens, Wisley, Ripley, Surrey.
- 1921 CHRYSTAL, R. N., D.Sc., Imperial Forestry Institute, Oxford.
- 1905 CORNWALLIS\*, F. S. W., Linton Park, Maidstone, Kent.
- 1915 COTTON, A. D., F.L.S., Royal Botanic Gardens, Kew, Surrey.
- 1920 CUNLIFFE, N., M.A., Research Institute, School of Forestry, Oxford.
- 1929 CURTIS, Miss K. M., M.A., D.Sc., Cawthron Institute, Nelson, New Zealand.
- 1920 CUTLER, D. W., M.A., F.L.S., Rothamsted Experimental Station, Harpenden, Herts.
- 1927 DADE, H. A., A.R.C.S., Mycologist, Research Branch, Department of Agriculture, Aburi, Gold Coast.
- 1920 DARBISHIRE, Prof. O. V., Ph.D., B.A., F.L.S., Botany School, The University, Bristol.
- 1915 DAVIDSON, J., D.Sc., F.L.S., F.E.S., The Waite Institute, University of Adelaide, S. Australia.
- 1927 DAVIES, W. MALDWYN, Ph.D., Adviser in Agricultural Zoology, University College, Memorial Buildings, Bangor, N. Wales.
- 1926 DAVY, J. BURTT, M.A., Ph.D., F.L.S., Imperial Forestry Institute, University of Oxford.
- 1923 DIXON\*, Miss A., M.Sc., F.R.M.S., Rothamsted Experimental Station, Harpenden, Herts.
- 1915 DOIDGE, Miss E. M., M.A., D.Sc., F.L.S., Division of Botany, Department of Agriculture, Pretoria, S. Africa.
- 1920 DOWSON, W. J., M.A., D.Sc., F.L.S., Department of Agriculture, Hobart, Tasmania.
- 1920 DRUMMOND, Prof. J. M., M.A., F.L.S., Botany School, The University, Glasgow.
- 1923 DU PORTE, ERNEST MELVILLE, M.Sc., Ph.D., F.E.S., F.R.M.S., Macdonald College, Montreal, Canada.
- 1925 DURHAM, H. E., Sc.D., M.B., B.C., F.R.C.S., "Dunedin," Hereford.
- 1927 EDWARDS, E. E., M.Sc., Advisory Entomologist, Harper Adams Agricultural College, Newport, Salop.
- 1925 EKINS, E. H., B.Sc., Principal, The College, Studley, Warwickshire.
- 1919 ELLIOTT, Mrs J. S. BAYLISS, D.Sc., Botany School, The University, Birmingham.
- 1917 ELTRINGHAM, H., M.A., D.Sc., F.E.S., 6, Museum Street, Oxford.
- 1922 ESDAILE, Miss P. C., D.Sc., F.Z.S., King's College for Women, Campden Hill Road, London, W. 8.
- 1927 EVERETT, J., B.A., Canterton Cottage, Lyndhurst, Hants.
- 1920 FAHMY, T., Mycological Division, Plant Protection Section, Ministry of Agriculture, Giza, Egypt.
- 1909 FARMER, Prof. Sir JOHN B., M.A., D.Sc., F.R.S., LL.D., Imperial College of Science, S. Kensington, London, S.W. 7.
- 1920 FENTON, E. W., M.A., B.Sc., F.E.S., Biological Department, Edinburgh and East of Scotland College of Agriculture, 13, George Square, Edinburgh.
- 1919 FISHER, K., The School, Oundle.
- 1923 FISHER, R. C., B.Sc., Ph.D., Forest Products Research Laboratory, Princes Risborough, Bucks.
- 1922 FREW, J. G. H., M.Sc., F.Z.S., F.E.S., 111, Mawson Road, Cambridge.

- 1919 FRYER, C. H., 4, Manor Grove, Dry Hill, Tonbridge.
- 1913 FRYER, J. C. F., M.A., F.E.S., Pathological Laboratory, Milton Road, Harpenden, Herts.
- 1919 FRYER, P. J., F.I.C., Messrs Wm. Cooper and Nephews (Australia), Ltd., 4, O'Connell Street, Sydney, Australia.
- 1918 GAHAN, C. J., M.A., D.Sc., F.E.S., Natural History Museum, S. Kensington, London, S.W. 7.
- 1914 GARDINER, Prof. J. S., M.A., F.R.S., Bredon House, Selwyn Gardens, Cambridge.
- 1927 GIBSON, Dr W. H., The Linen Industry Research Association, The Research Institute, Lambeg, Co. Antrim.
- 1922 GILCHRIST, Miss G. G., B.Sc., Botanical Department, The University, Bristol.
- 1920 GIMINGHAM, C. T., O.B.E., F.I.C., F.E.S., Pathological Laboratory, Milton Road, Harpenden, Herts.
- 1920 GLYNNE, Miss M. D., M.Sc., F.L.S., Department of Mycology, Rothamsted Experimental Station, Harpenden, Herts.
- 1920 GOODEY, T., D.Sc., Institute of Agricultural Parasitology, Winches Farm, Hatfield Road, St Albans.
- 1922 GOODWIN, W., M.Sc., Ph.D., S.E. Agricultural College, Wye, Kent.
- 1908 GOUGH, G. G., B.Sc., 45, Poplar Avenue, Edgbaston, Birmingham.
- 1921 GRAY, P. H. H., M.A., Rothamsted Experimental Station, Harpenden, Herts.
- 1905 GREEN, E. E., F.E.S., Way's End, Camberley, Surrey.
- 1909 GROOM, Prof. P., M.A., D.Sc., F.R.S., Imperial College of Science, S. Kensington, London, S.W. 7.
- 1921 GRUBB, N. H., M.Sc., East Malling Research Station, East Malling, Kent.
- 1921 GUILLEBAUD, W. H., B.A., Forestry Commission, 22, Grosvenor Gardens, London, S.W. 1.
- 1929 GURNEY, W. B., B.Sc., Entomologist, Department of Agriculture, Sidney, New South Wales.
- 1914 GÜSSOW, H. T., F.L.S., F.R.M.S., Dominion Botanist, Central Experimental Farm, Ottawa, Canada.
- 1920 GWYNNE-VAUGHAN, Prof. Dame HELEN, D.B.E., D.Sc., LL.D., F.L.S., Botanical Department, Birkbeck College, Chancery Lane, London, E.C. 4.
- 1920 HALKET, Miss A. C., B.Sc., Bedford College, Regent's Park, London, N.W.
- 1921 HARLAND, Prof. S. G., D.Sc., West Indian Agricultural College, Trinidad.
- 1924 HARRIS, R. V., Research Station, East Malling, Kent.
- 1927 HATTON, R. G., M.A., Director, East Malling Research Station, East Malling, Kent.
- 1920 HILEY, W. E., M.A., F.L.S., Research Institute, School of Forestry, Oxford.
- 1920 HILL, A. W., C.M.G., Sc.D., M.A., F.R.S., F.L.S., Director, Royal Botanic Gardens, Kew, Surrey.
- 1920 HISCOX, Miss E. R., B.Sc., Research Institute in Dairying, University College, Reading.
- 1924 HOCKEY, J. F., B.S.A., Pathologist in Charge, Plant Pathology Laboratory, Kentville, Nova Scotia, Canada.
- 1923 HODSON, W. E. H., A.R.C.S., D.I.C., Seale-Hayne Agricultural College, Newton-Abbot, Devon.

- 1920 HOLDEN, Prof. H. S., D.Sc., F.L.S., Department of Biology, University Park, Nottingham.
- 1919 HORNE, A. S., D.Sc., F.L.S., F.G.S., Botany School, Imperial College of Science, London, S.W. 7.
- 1920 HORTON, E., B.Sc., F.I.C., 10, Crieff Road, Wandsworth Common, London, S.W. 18.
- 1927 HOWES, F. N., M.Sc., Royal Botanic Gardens, Kew, Surrey.
- Orig. IMMS, A. D., M.A., D.Sc., F.L.S., F.E.S., Rothamsted Experimental Station, Harpenden, Herts.
- 1918 JACKSON, Miss D. J., F.L.S., F.E.S., North Cliff, St Andrews, Fife.
- 1927 JACOBS, S. E., Botanical Department, Imperial College of Science and Technology, S. Kensington.
- 1927 JAMES, H. C., D.Sc., c/o Agriculture Department, Scott Laboratories, Nairobi, Kenya, Africa.
- 1918 JARDINE, N. K., Royal Botanic Gardens, Peradeniya, Ceylon.
- 1928 JARRETT, Miss P. H., M.Sc., Department of Mycology, Rothamsted Experimental Station, Harpenden, Herts.
- 1927 JARY, S. G., Advisory Entomologist, University, Reading.
- 1907 JEPSON, F. P., M.A., F.E.S., Department of Agriculture, Peradeniya, Ceylon.
- 1927 JONES, G. H., M.A., Mycologist, Agricultural Department, Nigeria.
- 1927 JOSEPH, E. G., B.Sc., Imperial College of Science, S. Kensington, London, S.W. 7.
- 1919 KANNAN, KUHN, M.A., F.E.S., Assistant Entomologist, Government of Mysore, Bangalore, S. India.
- 1915 KEEBLE, Sir FREDERICK, C.B.E., M.A., Sc.D., F.R.S., c/o Nitram, Ltd., 28-30, Grosvenor Gardens, S.W. 1.
- 1920 KIDD, F., M.A., D.Sc., Low Temperature Research Station, Downing Street, Cambridge.
- 1907 KING, H. H., F.L.S., F.E.S., Government Entomologist, Wellcome Tropical Research Laboratories, Khartoum, Sudan.
- 1907 KING, Prof. L. A. L., M.A., West of Scotland Agricultural College, 6, Blythwood Square, Glasgow.
- 1926 KINGSTON, H. T., 8, Sewardstone Road, Waltham Abbey, Essex.
- 1924 KNIGHT, R.C., D.Sc., Assistant Director, Research Station, East Malling, Kent.
- 1921 LACEY, Miss M. S., D.Sc., Botanical Department, Imperial College, London, S.W. 7.
- 1921 LAURIE, Prof. R. D., M.A., F.Z.S., University College of North Wales, Aberystwith.
- 1908 LEES, A. H., M.A., National Fruit and Cider Institute, Long Ashton, Bristol.
- 1909 LEIPER, Prof. R. T., M.D., F.R.S., School of Tropical Medicine, Endsleigh Gardens, Euston Road, London, W.C. 1.
- 1926 LE PELLEY, R. H., Ph.D., School of Agriculture, Cambridge.
- 1915 LISTER, A. B., B.Sc., D.I.C., Experiment Station, Cheshunt, Herts.
- 1920 LLOYD, LLEWELLYN, D.Sc., Azare, Kano, Nigeria, Africa.
- 1914 McCLELLAN, F. C., C.B.E., M.R.A.C., F.L.S., Director of Agriculture, Zanzibar.
- 1928 McCULLOCH, R. F., B.Sc. (Agr.), School of Rural Economy, Oxford.

- Orig. MACDOUGALL\*, Prof. R. S., M.A., D.Sc., F.E.S., F.R.S.E., 9, Dryden Place, Edinburgh.
- 1925 MACLEOD, D. J., M.A., Officer in Charge, Dominion Plant Pathological Laboratory, Fredericton, New Brunswick.
- 1927 McLELLAN, Miss ETHEL, D.Sc., Botanical Department, The University, Melbourne, Australia.
- 1909 MANGAN, Prof. J., M.A., University College, Galway, Ireland.
- 1920 MANGHAM\*, Prof. S., M.A., Botany Department, University College, Southampton.
- 1917 MANN, H. H., D.Sc., F.L.S., 28, Ashburton Road, Oxtou, Birkenhead.
- 1914 MARSHALL, G. A. K., D.Sc., C.M.G., F.R.S., F.Z.S., F.E.S., Director, Imperial Bureau of Entomology, Natural History Museum, London, S.W. 7.
- 1927 MARTLEY, J. F., A.R.C.S., Forests Products Research Laboratory (Oxford Branch), c/o Imperial Forestry Institute, Oxford.
- 1922 MASON, E. W., M.A., M.Sc., Imperial Bureau of Mycology, 17, Kew Green, Surrey.
- 1920 MASON, F. A., 29, Frankland Terrace, Leopold Street, Leeds.
- 1921 MASON, T. G., M.A., Sc.D., Cotton Research Station, Trinidad.
- 1927 MASSEE, A. M., East Malling Research Station, East Malling, Kent.
- 1920 MATTICK, A. T. R., B.Sc., Research Institute in Dairying, University College, Reading.
- 1923 MILES, H. W., B.Sc., Department of Agricultural Entomology, University of Manchester.
- 1921 MILLARD, Prof. W. A., D.Sc., Department of Agriculture, The University, Leeds.
- 1928 MOORE, W. C., M.A., Plant Pathological Laboratory, Milton Road, Harpenden, Herts.
- 1922 MORLAND, D. M. T., M.A., Rothamsted Experimental Station, Harpenden, Herts.
- 1920 MORRIS, H. M., M.Sc., Agricultural Department, Nicosia, Cyprus.
- 1920 MOSLEY, F. O., F.L.S., Pathology Laboratory, Messrs Lowe and Shawyer, Uxbridge.
- 1927 MUIR, F. A. G., D.Sc., The Den, Farnham Common, Bucks.
- 1925 MUMFORD, E. PHILPOTT, B.Sc., Christ's College, Cambridge.
- 1914 MUNRO, J. W., M.A., D.Sc., Entomology Department, Imperial College of Science, S. Kensington, London, S.W. 7.
- 1919 MURPHY, A. J., 2, Dorset Square, London, N.W. 1.
- 1920 MURPHY, Prof. P. A., D.Sc., B.A., Albert Agricultural College, Glasnevin, Dublin, Ireland.
- 1925 MUSKETT, A. E., B.Sc., A.R.C.S., The Queen's University, Belfast.
- 1914 NEAVE, S. A., M.A., D.Sc., F.Z.S., F.E.S., Assistant Director, Imperial Bureau of Entomology, 41, Queens Gate, London, S.W. 7.
- 1927 NELSON, A., Ph.D., B.Sc., Superintendent of Research, Department of Agriculture, Hobart, Tasmania.
- 1927 NIRULA, R. L., B.Sc., Ph.D., D.I.C., Ghangwul, P.O. Jhawrian, District Shahpore, Punjab, India.
- 1923 NOEL, Miss E. F., 37, Moscow Court, London, W. 2.

- 1920 NOWELL, W., D.I.C., F.L.S., Director, Research Institute, Amani, Tanga, Tanganyika.
- 1923 OGILVIE, L., M.A., B.Sc., M.Sc., Research Station, Long Ashton, Bristol.
- 1925 OLDHAM, J. N., B.Sc., Institute of Agricultural Parasitology, Winches Farm, Hatfield Road, St Albans.
- 1923 OWEN, O., B.Sc., A.I.C., Experimental and Research Station, Cheshunt, Herts.
- 1919 PAINE, S. G., D.Sc., F.I.C., Imperial College of Science, London, S.W. 7.
- 1920 PALMER, R., "Standeford," Baldock Road, Letchworth.
- 1920 PARKER, T., Advisory and Research Department, Messrs Abol, Ltd., Beltring, Paddock Wood, Kent.
- 1914 PEARSON, J., D.Sc., Director of the Museum, Colombo, Ceylon.
- 1919 PERCIVAL, Prof. J., M.A., Sc.D., F.L.S., University, Reading.
- 1914 PETHERBRIDGE, F. R., M.A., School of Agriculture, Cambridge. -  
Orig. PETHYBRIDGE, G. H., M.A., M.R.I.A., Ph.D., B.Sc., Pathological Laboratory, Milton Road, Harpenden, Herts.
- 1919 POMEROY, A. W. J., Government Entomologist, Medical Research Institute, P.O. Box 300, Accra.
- 1915 PORTER\*, A., D.Sc., S. African Institute for Medical Research, P.O. 1038, Johannesburg.
- 1907 POULTON\*, Prof. E. B., M.A., D.Sc., LL.D., F.R.S., Wykeham House, Oxford.
- 1919 PRAIN\*, Sir DAVID, Lt.-Col., C.M.G., C.I.E., M.A., M.B., F.R.S., LL.D., F.R.S.E., V.M.H., The Well Farm, Warlingham, Surrey.
- 1923 PRESTON, N. C., B.Sc., Harper Adams Agricultural College, Newport, Salop.
- 1908 PRIESTLEY, Prof. J. H., D.S.O., B.Sc., Botany School, The University, Leeds.
- 1928 RAMSBOTTOM, J., O.B.E., M.A., British Museum (Natural History), Cromwell Road, London, S.W. 7.
- 1927 REGE, R. D., Ph.D., Institute of Plant Industry, Indore, Central India.
- 1921 RICHARDS, E. H., B.Sc., F.I.C., Rothamsted Experimental Station, Harpenden, Herts.
- 1921 ROACH, W. A., B.Sc., A.R.C.S., D.I.C., A.I.C., East Malling Research Station, East Malling, Kent.
- 1919 ROBERTS, A. W. RYMER, M.A., F.E.S., Molteno Institute for Research in Parasitology, Cambridge.
- 1923 ROBINSON, D. H., B.Sc., Harper Adams Agricultural College, Newport, Salop.
- 1916 ROBINSON, Prof. W., D.Sc., Stoneleigh, Caradoc Road, Aberystwyth.
- 1918 ROBSON, R., Institute of Agriculture, Chelmsford.
- 1920 ROEBUCK, A., N.D.A., Midland Agricultural College, Sutton Bonington, Derbyshire.
- 1919 RUSSELL, Sir JOHN, D.Sc., F.R.S., Director, Rothamsted Experimental Station, Harpenden, Herts.
- 1914 SALMON, Prof. E. S., F.L.S., S.E. Agricultural College, Wye, Kent.
- 1923 SAMUEL, G., B.Sc., University of Adelaide, S. Australia.
- 1920 SANDON, H., M.A., Rothamsted Experimental Station, Harpenden, Herts.
- 1921 SARGENT, R. H., Technical College, Darlington.
- 1919 SEARLE, G. O., B.Sc., Linen Industry Research Association, Glenmore House, Lambeg, Co. Antrim, Belfast.
- 1923 SINGH, BHAI INDER, 6, Tapp Road, Lahore, India.

- 1920 SMALL\*, Prof. J., D.Sc., F.L.S., Department of Botany, Queen's University, Belfast.
- 1920 SMITH, E. HOLMES, B.Sc., Botany School, The University, Manchester.
- 1925 SMITH, F. E. V., B.Sc., Microbiologist, Department of Agriculture, Kingston, Jamaica, British W. Indies.
- 1920 SMITH, J. HENDERSON, M.B., Ch.B., B.A., Department of Mycology, Rothamsted Experimental Station, Harpenden, Herts.
- 1920 SMITH, K. M., A.R.C.S., D.I.C., School of Agriculture, Cambridge.
- 1927 SMITH, Prof. N. J. G., M.A., B.Sc., Ph.D. (Camb.), Rhodes University College, Grahamstown (Cape), S. Africa.
- 1922 SNELL, Mrs E., Solent Court Farm, Warsash, Hants.
- 1913 SOUTH, F. W., M.A., Agricultural Department, Kuala Lumpur, Federated Malay States.
- 1928 STEER, W., B.A., East Malling Research Station, East Malling, Kent.
- 1925 STELL, F., Department of Agriculture, Port of Spain, Trinidad, British West Indies.
- 1919 SPEYER, E. R., M.A., Ridgehurst, Shenley, Herts.
- 1920 SPINKS, G. T., M.A., Research Station, Long Ashton, Bristol.
- 1923 STANLAND, L. N., A.R.C.S., D.I.C., Research Station, Long Ashton, Bristol.
- 1920 STAPLEDON, Prof. R. G., M.A., Agricultural Buildings, Alexandra Road, Aberystwyth.
- 1921 STENTON, R., F.E.S., Milton Road, Harpenden, Herts.
- 1922 STIRRUP, H. H., M.Sc., Midland Agricultural College, Sutton Bonnington, Loughborough, Derby.
- 1919 STONE, H., Forestry School, Cambridge.
- 1926 STOREY, H. H., M.A., Ph.D., East African Research Station, Amani, Tanga, Tanganyika.
- 1927 STOUGHTON, R. H., B.Sc., A.R.C.S., F.L.S., Department of Mycology, Rothamsted Experimental Station, Harpenden, Herts.
- 1921 SUTTON, E. C. F., Sidmouth Grange, Earley, near Reading.
- 1919 SUTTON, M. H., F.L.S., Erleigh Park, Whiteknights, Reading.
- 1905 SWANTON, E. W., A.L.S., Educational Museum, Haslemere, Surrey.
- 1919 TABOR, R. J., B.Sc., Botany School, Imperial College of Science, London, S.W. 7.
- 1921 TATTERSFIELD, F., D.Sc., F.I.C., Rothamsted Experimental Station, Harpenden, Herts.
- 1915 TAYLOR, H. V., A.R.C.S., B.Sc., Ministry of Agriculture, 10, Whitehall Place, London, S.W. 1.
- 1928 TAYLOR, T. H., M.A., Department of Agriculture, The University, Leeds.
- 1927 THAYSEN, A. C., Ph.D., Bacteriological Laboratory, Royal Naval Cordite Factory, Holton Heath, Dorset.
- 1928 THOMSON, W. S., B.A., Empire Marketing Board, 2, Queen Anne's Gate Buildings, London, S.W. 1.
- 1919 THORNTON, H. G., B.A., Rothamsted Experimental Station, Harpenden, Herts.
- 1920 TILLYARD, R. J., D.Sc., M.A., Sc.D., F.R.S., F.E.S., Commonwealth Entomologist, Canberra, Australia.
- 1927 TINCKER, M. A., M.A., M.Sc., Royal Horticultural Society's Gardens and Laboratory, Wisley, Ripley, Surrey.



- 1919 TROW, Principal A. H., D.Sc., F.L.S., University College, Cardiff.
- 1927 TURNER, W. H., B.Sc., Technical Department, Geo. Monro, Ltd., Waltham Cross.
- 1913 URICH, F. W., Imperial College of Tropical Agriculture, Trinidad, British West Indies.
- 1921 VOELCKER, J. A., M.A., B.Sc., Ph.D., F.I.C., 1, Tudor Street, London, E.C. 4.
- 1920 WAKEFIELD, Miss E. M., M.A., F.L.S., Royal Botanic Gardens, Kew, Surrey.
- 1923 WALKDEN, H., The Raft, Derbyshire Road, Sale, Manchester.
- 1919 WALLER, J. C., B.A., Physiological Department, The University, Liverpool.
- Orig. Warburton, C., M.A., Yew Garth, Grantchester, Cambridge.
- 1913 WARDLE, R. A., M.Sc., Zoological Department, The University, Manchester.
- 1919 WARE, W. M., B.Sc., S.E. Agricultural College, Wye, Kent.
- 1922 WARINGTON, Miss K., M.Sc., Rothamsted Experimental Station, Harpenden, Herts.
- 1914 WATERSTON, J., M.A., D.Sc., Natural History Museum, London, S.W. 7.
- 1920 WATT, A. S., B.A., Forestry Department, The University Botanic Gardens, Aberdeen.
- 1919 WEISS, Prof. F. E., D.Sc., F.R.S., F.L.S., Botany School, The University, Manchester.
- 1918 WEST, C., D.Sc., A.R.C.S., D.I.C., F.L.S., 7, Colfe Road, Forest Hill, London, S.E. 23.
- 1923 WESTON, W. A. R. DILLON, M.A., School of Agriculture, Cambridge.
- 1921 WHITEHEAD, T., D.Sc., A.R.C.S., University College of North Wales, Memorial Buildings, Bangor.
- 1912 WILLIAMS, C. B., B.A., Research Institute, Amani, Tanga, Tanganyika.
- 1920 WILLIAMS, Prof. R. STENHOUSE, M.B., C.M., B.Sc., D.P.H., Research Institute in Dairying, University College, Reading.
- 1927 WILLIAMS, T. L., B.A., A.I.C.T.A., Botanist, Agricultural Research Branch, Aburi, Gold Coast.
- 1909 WILLIAMSON, H. C., M.A., D.Sc., Pacific Biological Station, Namaimo, B.C., Canada. .
- 1919 WILLIS, J. C., M.A., Sc.D., F.R.S., F.L.S., 8, Cavendish Avenue, Cambridge.
- 1923 WILSON, Miss A. P., A.R.C.S., Horticultural College, Swanley, Kent.
- 1920 WILSON, G. FOX, R.H.S. Gardens, Wisley, Ripley, Surrey.
- 1914 WILSON, M., D.Sc., R.B.S., A.R.C.S., Royal Botanic Gardens, Edinburgh.
- 1921 WILTSHIRE, S. P., B.A., B.Sc., Assistant Director, Imperial Bureau of Mycology, 17, Kew Green, Kew, Surrey.
- 1921 WOODCOCK, G. S., address not known.
- 1926 WOODWARD, R. C., M.A., School of Rural Economy, Parks Road, Oxford.
- 1914 WORMALD, H., D.Sc., A.R.C.S., East Malling Research Station, East Malling, Kent.
- 1920 WORTLEY, E. J., F.I.C., M.B.E., F.C.S., Director of Agriculture, Zomba, Nyassaland.

## LAWS OF THE ASSOCIATION OF ECONOMIC BIOLOGISTS

I. The Association shall be called "The Association of Economic Biologists."

II. The object of the Association shall be to promote the study and advancement of all branches of Biology with especial reference to their applied aspects.

III. The Association shall consist of Ordinary and Honorary Members.

IV. Each candidate for ordinary membership shall be a subject of the British Crown. The nomination form of each candidate for ordinary membership shall bear the signatures of two members and shall be forwarded to the Secretaries. The nomination shall be submitted to the Council and, if approved, the election of the candidate shall be recommended to the Association at the next General Meeting. For the election of any candidate two-thirds of the votes of the members present and voting shall be cast in favour of the candidate.

V. All ordinary members on first election shall pay an entrance fee of half-a-guinea. Ordinary members shall pay an annual subscription of twenty-five shillings, due on January 1st of each year, or may compound for their subscriptions by payment of a sum of twenty-five pounds.

VI. Every member elected to the Association shall receive notice to that effect from the Secretaries and shall continue a member until his written resignation shall be received by the Secretaries, or until his membership be forfeited under the laws. (A member shall be liable for the annual subscription for the year in which his resignation takes effect and, notwithstanding his resignation, shall, if he so desires, receive any subsequent publications of the Association issued during that year.)

VII. Ordinary members shall be entitled to admission to all the meetings of the Association, to vote thereat, to present papers, to take part in discussions, and to receive a copy of the Association's publications. Each member shall be entitled personally to introduce non-members to any General Meeting of the Association. But no member whose subscription is in arrears shall be entitled to vote at a General Meeting or to receive the Association's publications, nor shall any publication be sent to a new member until his entrance fee and subscription shall have been received.

The Council may remove from the roll of the Association any member whose subscription is one year or more in arrears.

VIII. Honorary Members shall be persons, not subjects of the British Crown, who have contributed to an eminent degree to the advancement of the Science of Applied Biology. They shall be recommended by a majority of the whole Council and elected in the same manner as Ordinary Members. The number of Honorary Members shall not exceed twelve and not more than two shall be elected in any one year.

Honorary Members shall each receive a copy of the Association's publications and shall not be liable for the payment of an entrance fee or annual subscription.

Their privileges shall be the same as those of Ordinary Members except that they shall not be entitled to vote at any election or meeting of the Association.

IX. The business of the Association shall be conducted by a Council consisting of a President, a Treasurer, the Secretaries, of whom there shall be two; one representing the Botanical, the other the Zoological Sections of the Association, the Editors of the Journal, of whom there shall be two, and twelve Ordinary Members. Two members of the Council shall be nominated by the President to act as Vice-Presidents.

X. All properties of the Association, both present and future, shall be deemed to be vested in the Council of the Association for the time being, in conformity with the provisions of the Literary and Scientific Institutions Act, 1854.

XI. The Council shall meet at such times as they may determine; six members shall form a quorum.

XII. The Council shall have the power to fill any vacancies among its number that may occur other than those resulting from the selection for annual retirement from the Council referred to in Law XVII.

XIII. The Council shall have power, at any of their meetings, by two-thirds of the votes of those present and voting, to recommend the removal from the roll of membership of the name of any member for the reason that in their opinion it is contrary to the interests of the Association that he shall remain a member. Such recommendation shall be submitted to the Association at the next General Meeting. For the ejection from the Association of any member two-thirds of the votes of the members present and voting shall be cast in favour of such ejection.

XIV. The Council shall appoint a Publication Committee consisting of the Editors, the Treasurer, two Ordinary Members of the Council, and two Ordinary Members of the Association, who shall be responsible for the publication of the Journal of the Association.

XV. The Council, at a meeting prior to the Annual General Meeting, shall appoint one or more Auditors to audit the Treasurer's accounts.

XVI. The Council shall purchase such books, instruments, specimens, furniture and other necessities as may be required, pass the accounts and authorise their payment, and generally manage the affairs and administer the funds of the Association.

XVII. At a meeting prior to the Annual General Meeting the Council shall elect fourteen members of the existing Council and four members of the Association, not members of the existing Council, whom they recommend to the Association for election into the Council for the ensuing year. Any member of the Council vacating office shall not be eligible for re-appointment as an ordinary member of Council until after the lapse of twelve months. The list as drawn up by the Council shall be sent to all members resident in Great Britain and Ireland at least four weeks before the date of the Annual General Meeting. It shall be competent for any member, on receipt of the recommendations of the Council, to add the name of a member or members of the Association to the list of candidates for election to the Council; such additional nominations, duly seconded, must be in the hands of one of the Secretaries not less than fourteen days before the Annual General Meeting.

## 206 *Laws of the Association of Economic Biologists*

The Secretaries shall when necessary, and not less than seven days before the Annual General Meeting, issue to every member of the Association resident in Great Britain and Ireland a completed list of the proposed Officers and Council for the year, indicating the names of the proposers and seconders of candidates other than the Council's nominees.

The election of new Officers and Council shall be conducted in the following manner:—At the Annual General Meeting each member present shall receive the list of Officers and Council proposed for the year. If no additional nominations have been received after the Council's recommendations, the list shall be put to the meeting and voted on by a show of hands and the result declared by the Chairman. If additional nominations have been received a ballot shall be taken; each member voting shall hand in person to one of the Secretaries a copy of the list on which has been indicated the names of those candidates whom the member voting desires to serve on the Council. When the ballot has been declared closed the Chairman shall appoint from among the members present, two persons, not candidates for election, to serve as Scrutineers. In examining the lists handed in the Scrutineers shall set aside and take no account of any ballot paper which supports candidates for more than the number provided for in Law IX, nor of any ballot paper which indicates the identity of the member voting. The Scrutineers shall report to the Chairman of the meeting the result of their scrutiny, and the Chairman before the close of the meeting, shall announce the result of the ballot. In the case of an equality of votes for any candidates, the power of selection between them shall rest with the Chairman of the meeting and shall be exercised before announcing the result of the ballot.

XVIII. The Association shall meet at times and places to be decided by the Council.

At all Ordinary General Meetings ten shall form a quorum (see also Law XIX). All meetings shall be announced by circular addressed to each member resident in Great Britain and Ireland. At all Ordinary General Meetings the order of business shall be decided by the Chairman.

An Annual General Meeting shall unless otherwise decided by the Council be held at the date of the Ordinary General Meeting falling nearest to the beginning of the year.

At this General Meeting the order of business shall be: —

1. The reading of the minutes of the previous meeting.
2. The reading of a report of the Council on the work of the past year.
3. The statement of the Treasurer.
4. The election of Members.
5. The election of Officers and other Members of the Council.
6. Other business.

A Special General Meeting may be called to discuss or take action upon any matter affecting the interests of the Association.

A Special General Meeting shall be called either by the decision of the Council or at the request of at least ten members addressed to the Secretaries.

XIX. No new law shall be passed nor any standing law altered or added to, nor any other change in the constitution of the Association made except by a Special

General Meeting of which for this purpose a fourteen days' notice must be sent to all Members resident in Great Britain and Ireland.

The requisition for such a Special General Meeting duly signed and stating in writing the laws proposed or the alteration desired, must be delivered to one of the Secretaries, who shall within a reasonable period call such a meeting. The proposed new laws or alterations in the laws shall be printed in the circular convening the meeting.

At a Special General Meeting convened for the purpose of altering the constitution or amending the laws, fifteen shall form a quorum and no motion can be passed except by a two-thirds majority of those present and voting.



## STUDIES ON POTATO VIRUS DISEASES

## V. INSECT TRANSMISSION OF POTATO LEAF-ROLL

By KENNETH M. SMITH, D.Sc.

*(Potato Virus Research Station, School of Agriculture, Cambridge.)*

(With Plates X-XII.)

## CONTENTS.

	PAGE
1. Introduction . . . . .	209
2. Material and methods . . . . .	210
3. Infection tests in 1927 with different potato insects . . . . .	211
4. Infection tests in 1928 with <i>Myzus persicae</i> Sulz. . . . .	215
5. Non-transmission of the leaf-roll virus to the progeny of the aphid . . . . .	218
6. Effect on healthy potato plants of varying doses of virus . . . . .	219
7. Colonisation of infective aphides upon immune plants for varying periods before their subsequent transfer to healthy potatoes . . . . .	220
8. Separation by <i>M. persicae</i> of leaf-roll from a combination of two viruses . . . . .	222
9. Incubation period of the virus in the plant . . . . .	223
10. Infection of different varieties of potato with leaf-roll by <i>M. persicae</i> . . . . .	223
11. Feeding point of aphid in relation to infection of the host plant . . . . .	224
12. Passage of the virus through the plant, before development of leaf-roll symptoms . . . . .	225
13. Discussion . . . . .	225
14. Summary . . . . .	227
15. References . . . . .	228
Explanation of Plates . . . . .	229

## 1. INTRODUCTION.

IN many plant viruses there appears to exist an affinity between the virus and one particular insect which alone can disseminate it. This is illustrated by "Streak" of maize which is only transmitted by a certain species of leafhopper(7), and the "Yellows" disease of asters(2) where the same is true. It may be suggested that some such affinity exists between the virus of leaf-roll of potato and the aphid *M. persicae*, but evidence for this is not yet forthcoming. That it is the only carrier of leaf-roll is probably

not the case, and indeed other potato virus workers claim to have infected healthy plants by means of other widely differing insects (1, 4). There seems little doubt, however, in view of the evidence presented in the ensuing publication that *M. persicae* is the most efficient transmitter of leaf-roll, at all events under glasshouse conditions. The first object, then, of these studies, which was to discover an efficient insect vector of leaf-roll, may fairly be said to have been achieved, and the evidence for this is presented in the first half of this communication. The remainder of the paper is devoted to an account of some preliminary attempts to throw light upon the relations existing between virus and insect. The writer's thanks are due to Miss F. E. Hawkes for her kind assistance with the care of plants, and to Messrs F. Laing and F. V. Theobald for identifying aphides used in connection with this work.

## 2. MATERIAL AND METHODS.

Except for one experiment carried out in 1926 at the University of Manchester, all the work detailed in this communication was performed in the insect-proof glasshouse of the Potato Virus Research Station at Cambridge. The healthy "seed" used was partly from the writer's own stock grown for 3-4 years under insect-proof conditions and partly a number of tubers kindly given by Dr R. N. Salaman from his own stock of tested plants. The leaf-roll "seed" used for the infection of the insects was again derived partly from the writer's own stock which had been grown under insect-proof conditions for a number of years, and partly from leaf-roll tubers kindly supplied by Prof. Murphy of Dublin. The insects used, other than aphides, were collected from nettles in the vicinity of Cambridge; the aphides *M. gei* were collected from Iris sp. and the aphid *M. persicae* was from a stock, which the writer has had breeding for the past three years upon Cruciferae and other non-Solanaceous hosts.

Two methods of sprout infection were used, the first consisted in placing the sprouted half tuber in an ordinary 2 lb. glass jam jar, introducing the aphides on to the sprouts, and enclosing the top with a fine muslin cover held in place by a rubber band. The aphides failed to flourish under these conditions, and the method was abandoned for the following which proved satisfactory. The half tuber was planted in a 5 in. pot with the sprouts appearing above the soil, the aphides were then colonised upon the sprouts, and the whole covered with a glass lamp-chimney of the "hurricane" lamp type, the lower rim of the glass chimney afforded support when pushed into the ground, while a muslin cover held in place by a rubber ring round the upper rim of the glass



rendered the whole insect-proof. When the requisite time for infection had elapsed, the sprouts were cleared of aphides and the plant allowed to grow to maturity. This method gave the necessary temperature and humidity for the aphids, and a rapidly growing shoot in good condition for infection.

No sprout infection experiments of any kind were performed without half-tuber controls, and where infections were made on the growing plant either a tuber from the same parent or a cutting were grown as controls. Where no mention of the control plants is made it is implied that they remained healthy.

### 3. INFECTION TESTS IN 1927 WITH DIFFERENT POTATO INSECTS.

The following insects were used in the 1927 experiments:

#### HEMIPTERA.

##### HETEROPTERA.

- Capsidae.** *Calocoris bipunctatus* Fab. (*norvegicus*).  
*Lygus pabulinus* Linn.

##### HOMOPTERA.

- Jassidae** *Eupteryx auratus* Liv.  
(Leaf-hoppers). *Chlorita viridula* Fall.  
**Aphididae.** *Macrosiphum gei* Koch.  
*Myzus persicae* Sulz.

#### COLEOPTERA.

*Psylliodes affinis* Pk. Potato Flea Beetle.

#### HEMIPTERA. HETEROPTERA, Capsidae.

*Calocoris bipunctatus*. The infection experiments with this insect were divided into four series, each series consisting of six sprouted half tubers, with half tuber controls. The source of infection was a number of leaf-roll potatoes var. British Queen, and the half tubers used were known healthy Arran Victory. The capsids were collected in the vicinity of Cambridge from nettles only, and both adult and larval forms were used. Details, and results, of the inoculation tests with this insect are given in Table I.

A larger number of capsids per half tuber was found to be unsatisfactory, as the feeding punctures tended to destroy the sprouts.

Some experiments on the same lines as those carried out in the mosaic work (5) were also performed with this insect and leaf-roll. The salivary

Table I.

	Series 1	Series 2	Series 3	Series 4
Source of infection ... ..	Leaf-roll Brit. Queen	Leaf-roll Brit. Queen	Leaf-roll Brit. Queen	Leaf-roll Brit. Queen
Time on source ... ..	7 days	10 days	14 days	21 days
Time on exp. half tuber ...	7 days	17 days	14 days	12 days
Mean daily temp. ... ..	58° F.	69° F.	64° F.	68° F.
No. of capsids per half tuber	1	2	2	3
No. of capsids still alive at end of exp.	6	12	11	16
No. of half tubers inoculated	6	6	6	6
No. of plants infected ...	0	0	0	0

glands of a number of this species of capsid which had fed on a leaf-roll plant were extracted and inserted into the sprouts of half tubers. These all gave healthy plants. A further experiment was performed in which the faeces of capsids, bred on leaf-roll plants were inoculated into sprouting half tubers, this experiment was also negative.

*Lygus pabulinus*. Four similar series of sprout inoculations were performed with another species of capsid bug, *i.e.* *Lygus pabulinus* also from nettles (Table II).

Table II.

	Series 1	Series 2	Series 3	Series 4
Source of infection ... ..	Leaf-roll Brit. Queen	Leaf-roll Brit. Queen	Leaf-roll Brit. Queen	Leaf-roll Brit. Queen
Time on source ... ..	7 days	14 days	17 days	21 days
Time on exp. half tuber ...	10 days	14 days	14 days	11 days
Mean daily temp. ... ..	59° F.	64° F.	64° F.	68° F.
No. of capsids per half tuber	1	1	2	4
No. of capsids still alive at end of exp.	6	5	10	20
No. of half tubers inoculated	6	6	6	6
No. of plants infected ...	0	0	0	0

### HOMOPTERA. Jassidae (Leaf-hoppers).

*Eupteryx auratus*, *Chlorita viridula*. Two series of sprout inoculations were performed with the first of these, and one series with the second. It was necessary to use half tubers which had produced large leafy shoots before the hoppers could be induced to feed (Table III).

### Aphididae.

*Aphididae*. Two series of sprout inoculations were carried out with the aphid *Macrosiphum gei*, collected from Iris and other non-solanaceous host plants (Table IV).

Table III.

			Series 1 ( <i>E. auratus</i> )	Series 2 ( <i>E. auratus</i> )	Series 3 ( <i>C. viridula</i> )
Source of infection	...	...	Leaf-roll Brit. Queen	Leaf-roll Brit. Queen	Leaf-roll Brit. Queen
Time on source	...	...	9 days	14 days	10 days
Time on exp. half tuber	...	...	10 days	14 days	10 days
Mean daily temp.	...	...	60° F.	60° F.	60° F.
No. of leaf-hoppers per half tuber			3-4	4	4
No. of half tubers inoculated	...	...	6	6	6
No. of plants infected	...	...	0	0	0

Table IV.

			Series 1	Series 2
Source of infection	...	...	Leaf-roll Brit. Queen	Leaf-roll Brit. Queen
Time on source	...	...	Bred on leaf- roll plant	Bred on leaf- roll plant
Time on exp. half tuber	...	...	16 days	23 days
Mean daily temp.	...	...	60° F.	65° F.
No. of aphides per half tuber			12-18	50-100
No. of half tubers inoculated			6	6
No. of plants infected	...	...	0	0

The second species of aphid used was the small green aphid *Myzus persicae* Sulz. Six series of experiments were carried out with this insect—four sets of six sprouted half tubers, colonised with adult aphides, from leaf-roll British Queen, and two sets of six sprouted half tubers colonised with larval forms only, also from leaf-roll British Queen. Details of the experiments and the results achieved are set out in Table V.

Table V.

	Adult aphides				Larval forms	
	Series 1	Series 2	Series 3	Series 4	Series 5	Series 6
Source of infection	Leaf-roll Brit. Queen	Leaf-roll Brit. Queen	Leaf-roll Brit. Queen	Leaf-roll Brit. Queen	Leaf-roll Brit. Queen	Leaf-roll Brit. Queen
Time on source	Bred on l.-r. plant	Bred on l.-r. plant	Bred on l.-r. plant	Bred on l.-r. plant	Bred on l.-r. plant	Bred on l.-r. plant
Time on exp. half tuber	23 days	21 days	20 days	21 days	18 days	18 days
Mean daily temp.	63° F.	62° F.	63° F.	60° F.	63° F.	63° F.
No. of aphides per half tuber	12-18	12-18	12-18	12-18	12-18	12-18
No. of half tubers inoculated	6	6	6	6	6	6
No. of plants infected	6	6	6	6	4	4

Out of the 24 half tubers inoculated with adult infective aphides, 24 infected plants were produced; and out of 12 half tubers inoculated with the larval forms, 10 infected plants were produced. This shows the larval forms to be efficient carriers of leaf-roll, provided they have fed on a leaf-roll plant sufficiently long to pick up the virus. In the above experiments symptoms developed in an average period of 32 days from the first date of infection.

### COLEOPTERA.

*Psylliodes affinis*. Potato Flea Beetle. One series only of experiments was carried out with this insect. With this beetle as with the leaf-hopper it was necessary to use half tubers with large leafy sprouts, in order that the insect might be induced to feed (Table VI).

Table VI.

Source of infection ...	...	Leaf-roll Brit. Queen
Time on source ...	...	24 days
Time on exp. half tuber ...	...	12 days
Mean daily temp. ...	...	68° F.
No. of beetles per half tuber		2
Results ...	...	All plants healthy

It is realised that such a limited experiment as the above can only be regarded as a preliminary test of the leaf-roll carrying powers of this insect.

It will be seen from the foregoing tables of sprout infections, that out of the seven different insects tested only one, the aphid *M. persicae*, gave positive results. This aphid, as later experiments will show, has proved itself to be a most efficient carrier of leaf-roll. The progeny of the plants arising from the experimental half tubers used in all these tests were grown the following year (1928). All produced healthy plants with the exception of the thirty-four successful inoculations with *M. persicae* which gave leaf-roll plants (Plate X, fig. 2).

#### *Haulm Infection Experiments in 1927 with Eupteryx auratus.*

As it was found difficult to induce this leaf-hopper to live satisfactorily upon the sprouts of the tuber, three series of infections of the haulm were carried out. In the second series of experiments, the leaf-hoppers used were the progeny of a number of leaf-hoppers which had been allowed to breed on a leaf-roll plant. These insects, during their lifetime therefore, had fed only upon leaf-roll potato foliage (Table VII).

Table VII.

	<i>Series 1</i>	<i>Series 2</i>	<i>Series 3</i>
Source of infection ...	Leaf-roll Brit. Queen	Leaf-roll Brit. Queen	Leaf-roll Brit. Queen
Time on source ...	14 days	Bred	Bred
Time on exp. plant ...	18 days	16 days	13 days
Mean daily temp. ...	65° F.	67° F.	Out of doors insectary
No. of plants inoculated	6	6	6
No. of plants infected ...	0	0	0

All these plants remained healthy throughout 1927. The progeny were grown in the field during 1928. On July 26th, 1928, it was noticed that three plants showed leaf-roll; there is therefore a possibility that the leaf-hopper had infected the parent plants the year before. The writer however is more inclined to the view that the disease resulted from external infection by *M. persicae* in the field during the summer of 1928, especially as the three plants infected were not all the progeny of one parent but isolated plants.

#### 4. INFECTION TESTS IN 1928 WITH *MYZUS PERSICAE* SULZ.

Following upon the successful sprout inoculations with this insect in 1927, further inoculation tests were performed in 1928. The first four series consisted each of twelve sprouted half tubers, var. Arran Victory, with half tuber controls; these were colonised in glass jars and later cleared of aphides and potted up. The fifth series, consisting of six half tubers, var. President, were infected by means of the improved method already described, where the half tuber is planted in a pot and covered with a glass lamp chimney. This method was adopted for all further sprout inoculations with insects. In the first two series of sprout infections the immature forms only of the aphid *M. persicae* were used. The sprouts were infected in the glasshouse on February 29th, on March 20th the sprouts were cleared of aphides and potted up. Symptoms of leaf-roll appeared on April 16th, 36 days after the first infection of the sprouts with the virus-carrying aphides. By the end of April 20 plants out of the 24, arising from the experimental half tubers, had developed leaf-roll, the other four remained healthy. On March 21st the two further series of experiments with Arran Victory were performed; this time adult aphides were used and were allowed to feed for 28 days before the sprouts were cleared. The half tubers were planted on April 27th; nine days later one of the plants, now a few inches above ground, showed slight symptoms of leaf-roll. By May 16th all 24 plants had developed leaf-roll (see Plate X, fig. 4). The

fifth series, six half tubers var. President, were colonised with aphides on April 19th, these were cleared of aphides 14 days later, May 3rd, by May 25th all six plants were leaf-rolled (Plate XI, figs. 1-3). In one case the plant developed symptoms soon after its appearance above ground. Details of these sprout inoculations are given in Table VIII.

Table VIII.

1928 Sprout Inoculations with *Myzus persicae*.

	Series 1	Series 2	Series 3	Series 4	Series 5
Commencement of experiment	Feb. 29th	Feb. 29th	March 21st	March 21st	April 19th
Date of appearance of first symptoms	April 16th	April 20th	May 6th	May 9th	May 20th
Variety of half tubers	Arran Victory	Arran Victory	Arran Victory	Arran Victory	President
Source of infection		Leaf-roll	British Queen in each case		
Time on source	Bred	Bred	Bred	Bred	Bred
Time on exp. half tuber	20 days	20 days	28 days	28 days	14 days
Mean daily temperature	62° F.	62° F.	65° F.	65° F.	65° F.
No. of aphides per half tuber	6	6	12-18	12-18	12-18
Description of aphides used	larvae	larvae	adults	adults	adults
No. of half tubers used	12	12	12	12	6
No. of plants infected with leaf-roll	10	10	12	12	6

1928 Haulm Inoculations with *Myzus persicae*.

Two series of infections of the haulms of young growing plants were carried out with *M. persicae* infected with the virus of leaf-roll. The experiments were performed under canvas cages in one of the compartments of the insect-proof glasshouse. The first series consisted of six young Arran Victory plants, which were colonised with the infective aphides on March 6th. On April 11th the first symptoms of leaf-roll appeared, and by the end of the month five out of the six plants had developed the disease (see Plate XI, fig. 6). The sixth plant which was poorly colonised with the aphids remained healthy.

In the second series six young plants var. President were used, and colonised with aphides on April 2nd. The first symptoms of leaf-roll appeared on April 30th, by the middle of May all six plants had developed leaf-roll. A third experiment was performed in the open air insectary as follows: a leaf-roll British Queen plant on which a number of *M. persicae* were feeding, was placed near to, but not touching, six young Arran

Victory plants of known healthy stock. No aphides were transferred from the leaf-roll plant but were allowed to breed untouched. After about six weeks one of the healthy Arran Victory plants developed leaf-roll to be followed shortly after by a second, showing that infection had been carried by migration of the aphid from the leaf-rolled plant. The experiment was then discontinued. The large number of positive infections obtained under controlled conditions by means of *Myzus persicae*, detailed in the foregoing experiments, are sufficient in the writer's opinion to incriminate this aphid as an efficient vehicle for the dissemination of leaf-roll.

Although entirely negative results were obtained in the inoculation experiments with the other potato feeding insects, it is unwise to deduce on that account that such insects are unable under any circumstances to disseminate leaf-roll. Suffice it to say that so far they have not done so, under conditions giving positive infections with *Myzus persicae*. Other potato virus workers, Murphy (4) and Elze (1), are of the opinion that some at least of these insects are capable of transmitting leaf-roll.

The development of leaf-roll in the variety Arran Victory after inoculation by *M. persicae*, which has been closely studied by the writer, exhibits a fairly consistent sequence of symptoms. The first signs of the disease (primary leaf-roll) appear about thirty days after the date of insect colonisation; they consist first of a general pallor of the upper and youngest leaves followed later by a rolling starting from the base and usually accompanied by a well-marked pigmentation, brownish-black in colour, which is characteristic of leaf-roll in this variety. Under glass-house conditions, this primary leaf-roll rapidly passes into the secondary form in which the lower leaves show a marked interveinal pallor, becoming leathery and harsh to the touch. They then begin to roll, usually developing at the same time the brownish-black pigmentation which may occupy the whole base of the leaf, as shown in Plate XI, fig. 5. The whole plant is stunted, often presenting a purplish tint, and in a bad case every leaf may be rolled. In Arran Victory a tendency to form aerial tubers is often associated with leaf-roll. Half tubers inoculated by means of *M. persicae* at the end of February and the beginning of March produced plants with well marked secondary leaf-roll within two months of that date.

These plants differed in no degree of severity from the progeny of plants infected with leaf-roll by the aphid in the preceding year (Plate X, fig. 2). In the glasshouse the half tubers infected by *M. persicae* with leaf-roll produced plants which averaged from 9-12 in. in height and gave a negligible yield of tubers, while the control half tubers grown under the

same conditions but without insect inoculation produced plants which averaged 5 ft. in height and gave a good crop of tubers.

Sufficient evidence of the power of this aphid to transmit leaf-roll having been offered, some further investigations into the part played by this insect in the dissemination of leaf-roll are described.

#### 5. NON-TRANSMISSION OF THE LEAF-ROLL VIRUS TO THE PROGENY OF THE APHIS.

Experiments to determine this somewhat important point were carried out during the years 1927–1928. In 1927 the experiments were planned as follows: 25 mature viviparous female *M. persicae*, which had been bred on a leaf-roll British Queen plant, were placed each one in an empty glass-bottomed pill box. As each parthenogenetically produced young appeared, it was picked up with a fine camel's hair brush and placed upon a sprouted half tuber var. Arran Victory with half tuber control. All the young produced by each female aphid were kept together on their respective half tubers. When sufficient young aphides, 6–9, had been produced, the mother aphid was placed on a sprouted half tuber in order to demonstrate that she herself was infective. This precaution was taken because the writer is of the opinion that the virus-carrying powers may differ in individual aphides of this species. By the pill box method described above (first used by Murphy in Dublin<sup>(4)</sup>) all fear of contamination of the young aphid from other sources is eliminated. Thus far the experiment consists, then, of 25 sprouted half tubers each with one adult female aphid, with 25 half tuber controls, and 25 half tubers each with 6–9 young aphides and 25 controls, each set of young corresponding to one female aphid. When the young of the first generation were mature, they in their turn were placed in pill boxes, and the young transferred to a further 25 sprouted half tubers with controls. This was to investigate the possibility of inheritance of the virus to the second generation. The 150 half tubers constituting the whole experiment were grown in the insect-proof glasshouse under identical conditions, all remained healthy with the exception of 5 (20 per cent.) of the plants produced by those half tubers which had been colonised with the original virus-carrying females. This experiment was therefore negative as regards the inheritance of the virus by the aphid progeny; one point however arises, *i.e.* that one aphid is capable of infecting a plant with leaf-roll. The facts that *M. persicae* tends to die easily when away from any food plant for more than 24 hours, and also that under starvation conditions it does not reproduce readily, rendered the experiment somewhat difficult to perform owing to



the necessity of replacing the adults which constantly died without reproducing. In 1928 the work was simplified, ten half tubers only were colonised with the young from adult females placed in glass boxes as before, and the second generation was not considered. This gave a total of 20 experimental plants and 20 controls; of the ten plants arising from the half tubers colonised with the adult aphides five were infected with leaf-roll, while the ten infected with the young aphides remained healthy. So far as negative evidence can be conclusive, these experiments carried out over two years, indicate that the virus is not inherited by the progeny of an infective aphid. This conclusion is in agreement with the findings of workers on other insect-carried plant viruses, and the writer is not aware of an authoritative case of such inheritance other than that quoted in the work of McClintock and Smith (3) on spinach blight.

#### 6. EFFECT ON HEALTHY POTATO PLANTS OF VARYING DOSES OF VIRUS.

The object of this experiment was to ascertain whether the *number* of infective aphides attacking a plant was of importance, and whether the plant would react differently to the varying amounts of virus received in consequence. Sprouted half tubers were used (var. Arran Victory) and six series of experiments were performed, each one consisting of four inoculation tests, *i.e.* 2, 6, 12 and 18 aphides respectively per half tuber. The aphides used for each experiment had all been bred on the same leaf-roll plant, and were as far as possible uniform as to size, age, etc. Details of the experiments are given in the table. The results show that the incidence of infection is greatest among those plants colonised with 12 and 18 aphides. Nevertheless infection was produced among the plants colonised with two aphides only, and when achieved the disease differed in no way from that produced by 18 aphides. This is in agreement with Storey's findings with streak disease of maize (7). The small incidence of infection among the plants treated with two and six aphides may be explained partly by the fact that *M. persicae* is very sensitive to environment, and if conditions of temperature and humidity are not suitable, it tends to leave its food plant and wander away, and in an experiment of this kind obviously the aphides could not be replaced without risk of nullifying the experiment. Another possible explanation is variability in the infective power of individual aphides (Table IX).

Table IX.

	Experiment 1				Experiment 4			
	2 aphides	6 aphides	12 aphides	18 aphides	2 aphides	6 aphides	12 aphides	18 aphides
Date of experiment	May 14	May 14	May 14	May 14	May 21	May 21	May 21	May 21
First appearance of symptoms	Healthy	Healthy	June 12	June 12	June 21	June 21	June 18	June 15
Incubation period in plants	—	—	29 days	29 days	31 days	31 days	28 days	25 days
	Experiment 2				Experiment 5			
Date of experiment	May 16	May 16	May 16	May 16	June 15	June 15	June 15	June 15
First appearance of symptoms	June 19	June 18	June 18	June 12	Healthy	Healthy	July 12	July 12
Incubation period in plants	34 days	33 days	33 days	27 days	Healthy	Healthy	27 days	27 days
	Experiment 3				Experiment 6			
Date of experiment	May 18	May 18	May 18	May 18	July 1	July 1	July 1	July 1
First appearance of symptoms	Healthy	Healthy	June 18	June 20	Healthy	Healthy	Healthy	Aug. 5
Incubation period in plants	—	—	31 days	33 days	Healthy	Healthy	Healthy	35 days

#### 7. COLONISATION OF INFECTIVE APHIDES UPON IMMUNE PLANTS FOR VARYING PERIODS BEFORE THEIR SUBSEQUENT TRANSFER TO HEALTHY POTATOES.

Experiments were performed to determine the effect produced on the infective power of *M. persicae* by allowing the virus-carrying aphides to feed upon immune non-solanaceous plants before transferring them to healthy potatoes. It was considered possible that prolonged feeding upon immune plants might have the effect of clearing the virus from the body of the aphid and thus render it non-inoculative, especially if the insect be a mechanical carrier of the virus. The immune plant selected for this work was the cabbage and the experiments were performed as follows: aphides which had been bred upon leaf-roll British Queen potato plants were allowed to feed on cabbage for 24, 48, and 72 hours, and 7 days respectively; they were then transferred, each lot, to three sprouted half tubers var. Arran Victory. At the same time a number of the same stock of infective aphides were transferred directly to three sprouted half tubers var. Arran Victory, to demonstrate that this particular lot of aphides was actually carrying the virus. The results were as follows: the three half tubers colonised with aphid direct from the leaf-roll plant produced plants which developed the disease 22 days later; the three half tubers colonised with aphid after 24 hours on cabbage gave rise to diseased plants 23 days later, those with aphid 48 and 72 hours on cabbage, 22 and 18 days later

respectively, and those with aphid 7 days on cabbage, 26 days later (Plate XII, Fig. 1). The experiment was then repeated using a different set of *M. persicae* from another leaf-roll plant, and omitting the direct infection of potato and the 72-hour period on cabbage. The results were the same as in the preceding experiment—two out of the three plants in the 24 hour experiment developed leaf-roll in 21 days, the third plant remained healthy. The 48 hours and 7 days period experiments all developed the disease in 22 and 20 days respectively. From these results it will be seen that feeding upon a non-susceptible plant host has no effect upon the infective power of the aphid. It seems probable, but this has not yet been proved, that *Myzus persicae* once infected remains so for the rest of its life. With so prolific an insect as the aphid it is a difficult matter to keep individual insects under observation for more than a week, rapid multiplication rendering it difficult to trace the original insects used. In order therefore to feed infective aphides upon cabbage for longer periods than a week, it would be necessary to pick off the young each day as born, and where large numbers of aphides are employed this is no small undertaking. Details of the experiment are given in Table X. An investigation into

Table X.

*Colonisation of infective aphides upon immune plants  
before their transfer to healthy potatoes.*

<i>Experiment 1.</i>					
	Direct transfer to healthy potatoes	24 hours on cabbage	48 hours on cabbage	72 hours on cabbage	7 days on cabbage
Date of experiment	May 30th	May 30th	May 30th	June 18th	May 30th
Date of first symptoms	June 19th	June 22nd	June 21st	July 6th	June 26th
No. of half tubers inoculated	3	3	3	3	3
No. of plants infected with leaf-roll	3	3	3	3	3
<i>Experiment 2.</i>					
Date of experiment	—	June 16th	June 16th	—	June 18th
Date of first symptoms	—	July 6th	July 7th	—	July 9th
No. of half tubers inoculated	—	3	3	—	3
No. of plants infected with leaf-roll	—	2	3	—	3

the effect of starvation upon the transmitting powers of *M. persicae* was also carried out. It was found that aphides remained infective after

4-5 days' starvation, about the maximum period without food to which this aphid can be subjected.

#### 8. SEPARATION BY *M. PERSICAE* OF LEAF-ROLL FROM A COMBINATION OF TWO VIRUSES.

Some preliminary experiments were performed to determine the virus-carrying powers of *M. persicae* after feeding upon a potato plant affected with a combination of two viruses of which one was leaf-roll. Two sets of experiments were carried out: (a) with a leaf-roll streak combination, (b) with leaf-roll mosaic.

In the first case the leaf-roll streak combination was obtained as follows: an Up-to-Date plant which was known to be "carrying" streak, and which had been tested by grafting on to Arran Victory, a variety very susceptible to streak, was infected early in the season with leaf-roll by means of *M. persicae*. It is perhaps worthy of mention that infection of the Up-to-Date plant with leaf-roll seemed to cause the appearance of streak symptoms hitherto suppressed (Plate XII, fig. 2). This plant, then, showing plainly the symptoms of both diseases was colonised with *M. persicae*, which later were transferred in the usual way to six sprouted half tubers var. Arran Victory, and three half tubers var. President. Symptoms of leaf-roll developed in two Arran Victory plants in 31 days and in two more Arran Victory plants in 37 days. The three President and the two remaining Arran Victory plants remained healthy. Although both President and Arran Victory are exceedingly susceptible to streak and both varieties had actually been infected from this same plant by grafting, no symptoms of this disease appeared, thereby further confirming the writer's inability to induce any insect to transmit streak as such from diseased to healthy plants. The results of this experiment indicate that *M. persicae* will pick up the leaf-roll virus from a combination of streak and leaf-roll and leave the streak behind, or perhaps it would be more correct to say that the plant inoculated by the aphid from the streak leaf-roll plant apparently receives only leaf-roll.

The leaf-roll mosaic combination in the second experiment was obtained by infecting a mosaic Arran Victory with leaf-roll by means of *M. persicae*. When the plant had developed leaf-roll it was colonised with *M. persicae* for transfer to healthy half tubers. In this experiment an attempt was made to discover whether the aphid was picking up both viruses; in a previous publication<sup>(5)</sup> the writer has shown that the tobacco plant is a sensitive indicator to potato mosaic carried by *M. persicae*. Aphides from the leaf-roll mosaic Arran Victory were therefore

colonised on healthy sprouted half tubers, and also upon a number of healthy tobacco seedlings (var. White Burley). The results of this experiment were as follows: the half tubers of healthy Arran Victory produced leaf-roll plants, first symptoms developing after 25 days. The tobacco plants which do not react to the leaf-roll virus developed the typical mottle disease which mosaic-bearing *M. persicae* produce on tobacco<sup>(5)</sup>. This shows then that the aphid apparently picks up both viruses and that the separation of them depends more upon the plant than the insect. The above are preliminary experiments only and further work on these lines is contemplated.

#### 9. INCUBATION PERIOD OF THE VIRUS IN THE PLANT.

The incubation period of the leaf-roll virus in the plant, counting from the first day of colonisation of the sprouted half tuber with the aphid, varies between 18–50 days, with an average period of 30 days at a mean daily temperature of 65° F. The periods of 40–50 days only occurred in those experiments where the infective aphides were allowed to remain on the unplanted half tubers for 20 days or more before the latter were potted up. Infection with leaf-roll by means of *M. persicae* is therefore usually more rapid than by means of grafting, presumably because the period of waiting for union of scion and stock is eliminated. The question of the incubation of the virus in both plant and insect is still under investigation. Experiments are in progress which are designed to show with considerable accuracy the duration of the incubation period of the virus in the plant, the incubation period, if any, of the virus in the body of the insect, and the time necessary for the insect to feed upon an infected plant in order to pick up the leaf-roll virus.

#### 10. INFECTION OF DIFFERENT VARIETIES OF POTATO WITH LEAF-ROLL BY *M. PERSICAE*.

With a view to ascertaining whether any difference in degree of resistance to leaf-roll existed in different varieties of potatoes, inoculations with *M. persicae* were made with the following varieties: President, King Edward, Arran Chief, Kerr's Pink and Great Scot, to which may be added Arran Victory so largely used in the experimental work, and Edzell Blue which was experimentally infected in 1926. All these varieties developed leaf-roll in the current season. The tubers used in this experiment were tested healthy stock, and the infective aphides were colonised on sprouted half tubers with the usual half tuber controls. Some details

of the development of the leaf-roll symptoms in the different varieties are given.

*President.* The first sign of the disease in this variety is a pallor at the edges of the young leaves, later this spreads to the lower leaves which become much thickened and leathery. There is less actual rolling of the leaves in President than in Arran Victory. The whole tendency of a leaf-roll President plant is towards a stiff upright habit. Often the young leaves show a pale yellowish coloration on the upper side with an accumulation of pink pigment on the lower (Plate XI, fig. 4). The whole plant is very harsh to the touch and rattles when shaken (Plate XI, figs. 1-3).

*Great Scot.* Six half tubers of this variety were colonised with infective aphides on June 22nd; first signs of leaf-roll appeared on one plant on July 12th, 20 days later. By July 26th all six plants were infected. Actual rolling in this variety does not appear to be very pronounced, first symptoms appeared as a marked interveinal pallor, accompanied by a stiffness of the lower leaves, later some rolling develops (Plate XII, fig. 4).

*King Edward.* Six half tubers were colonised with infective aphides on July 4th, symptoms of leaf-roll developed in one plant on July 31st, 27 days later. All plants developed leaf-roll by August 14th. A general pallor first appeared on the young leaves followed by a slight rolling at the base. Pink coloration developed on the lower surface of both young and old leaves, later fairly pronounced rolling appeared on the lower leaves accompanied by the usual stiffness and leatheriness (Plate XII, fig. 5).

*Arran Chief.* Six half tubers infected on July 2nd produced diseased plants on July 31st, 29 days later. First symptoms appeared as a marked interveinal pallor mostly on the young leaves, later rolling developed accompanied by some pink pigmentation, the leaves becoming yellowish on the upper surface, stiff and harsh.

*Kerr's Pink.* Six half tubers were infected on June 28th, first symptoms appeared on August 2nd. The young leaves developed an interveinal pallor accompanied by rolling at the base with some pink pigmentation. Three plants only developed current season symptoms.

#### 11. FEEDING POINT OF APHIS IN RELATION TO INFECTION OF THE HOST PLANT.

It has already been conclusively demonstrated that the leaf-roll virus is distributed by infective aphides feeding either upon the sprouts of the tuber, or upon the leaves of a young growing plant, although infection

may take longer to develop when the aphid feeds on the haulm. An experiment was performed to determine whether the plant was as easily infected when the aphid fed only upon the stem. Three half tubers var. President were planted when the resulting plants were five inches high, all the leaves were removed. Infective aphides were then colonised on the bare stem; the new shoots arising from the other eyes of the half tubers developed leaf-roll when about three inches high, 34 days after colonisation with the aphides.

As would perhaps be expected, this experiment shows that infection with leaf-roll is as easily brought about by aphid punctures in the stem as by punctures in sprout or leaf.

#### 12. PASSAGE OF THE VIRUS THROUGH THE PLANT, BEFORE DEVELOPMENT OF LEAF-ROLL SYMPTOMS.

In this experiment a number of half tubers were used in which all the eyes had been removed save two, diagonally opposite each other. One shoot was colonised with infective aphides, the other was kept uninfected. Leaf-roll appeared simultaneously in both shoots showing that the virus had passed down the infected shoot and through the half tuber into the uninfected shoot before the symptoms of the disease developed.

#### 13. DISCUSSION.

A number of potato-feeding insects mostly of the plant-sucking Hemiptera have been tested as to their capabilities for transmitting leaf-roll. The aphid *Myzus persicae* Sulz. is outstanding as a most reliable disseminator of this disease. Although tests with the other insects proved negative, much more work remains to be done with those insects before they can be regarded definitely as incapable of transmission. That *M. persicae* is a more efficient carrier of the virus can hardly be doubted, and it is probable that this insect is responsible for much of the spread of leaf-roll in the field. In the glasshouse it is possible to transmit leaf-roll to a healthy plant by means of *M. persicae* with almost the same reliability as grafting and in a shorter time. The disease first appears in its "primary" form where the young leaves are affected (Plate X, figs. 1 and 4), but this very rapidly passes, at all events under glasshouse conditions, into the "secondary" condition where the whole plant is affected (Plate X, figs. 5 and 6).

As regards the other experiments described in this paper, some points of interest have arisen. Two years' work on the question of the heredity of the leaf-roll virus by the progeny of the aphid has given negative

results, and it may fairly be concluded that the virus is not inherited; this is in accord with the findings of most workers upon insect-borne plant viruses. It has also been shown that two individuals of *M. persicae*, and in a few cases one, are capable of infecting a plant with leaf-roll, the disease thus produced differing in no degree of severity from that produced by 18 infective aphides. That the aphid is no mechanical carrier in the sense that the virus adheres merely to the external mouthparts is shown by the fact that periods up to seven days' feeding on immune plants such as cabbage are no obstacle to the subsequent infection of healthy potato plants, though this fact cannot be taken as evidence of a direct relationship between virus and insect. The whole question of such relationship, if one exists, between the aphid and the leaf-roll virus is one of great interest, but more evidence is still required on this point. That it is possible for two distinct viruses to exist together unchanged in the body of the aphid is suggested by the preliminary experiments on the transmission of combinations of leaf-roll and mosaic. By placing the aphides, after feeding upon a plant with this combination, upon other plants which react differently to the two viruses, *i.e.* tobacco and potato, it was found that the former developed mosaic and the latter leaf-roll. This appears to show that whatever virus or viruses be present in a plant they are picked up by the aphid and passed on to the next host plant, which develops the disease to which it may be most susceptible, in this case the tobacco is most susceptible to potato mosaic and the potato to leaf-roll. As to why *M. persicae* is a more efficient carrier of leaf-roll than other insects, it is difficult to say in the present state of knowledge of this subject. It may be due to the fact that leaf-roll is primarily a phloem disease, and as the writer has shown (6) *M. persicae* is much more a phloem feeder than many of the other Hemiptera, though not more than other aphides. This explanation hardly seems to be the correct one in view of the facts that it is not possible to inoculate a potato, by means of the needle, with leaf-roll but it is with mosaic, and if it were a question, merely, of reaching the phloem with the virus, that could surely be accomplished with the needle. It may be that passage of the leaf-roll virus through the body of the aphid increases its infective power or attunes it in some way to reception by the plant. It has been shown (5) that the virus of potato mosaic transmitted to tobacco by the aphid produces a disease in which the symptoms differ from those exhibited by the disease arising from needle inoculations into tobacco with the same virus. This may be a case where the virus has changed slightly in the body of the aphid, or again it may be that difference in symptoms arises from the difference in the mode of



inoculation. So far as the experiments on aphid infection of varieties go, no very marked difference in degree of resistance to leaf-roll was apparent, all the varieties tested developing leaf-roll within the current season. The incubation period of the virus in the plant is usually about 30 days, counting from the first day of colonisation with the infective aphides. As with most plant viruses a condition of rapid growth and movement is necessary for infection with leaf-roll, and it was found increasingly difficult as the season advanced to obtain successful inoculations with the aphides.

The actual feeding point of the aphid on the plant makes little difference to ultimate infection; it is possible for the aphid to disseminate the leaf-roll virus by feeding upon the sprouts of the tuber, the haulm of the plant or upon the stem itself.

#### 14. SUMMARY.

1. Inoculation experiments with seven different species of insects carried out in 1927 gave negative results, except in the case of the aphid *Myzus persicae* Sulz. which gave a high percentage of positive infections.

2. Further inoculation tests with this aphid in 1928 proved it to be an efficient carrier of the leaf-roll virus.

3. Experiments carried out during two years on the question of the inheritance of the leaf-roll virus by the progeny of infective aphides proved negative. It is therefore assumed that the virus is not hereditary in the offspring of the aphid.

4. One or two virus-bearing aphides are capable of infecting a healthy potato plant with leaf-roll. Such infection when achieved differed in no degree of severity from that induced by 18 aphides. The incidence of infection was greatest among plants colonised with groups of 18 infective aphides.

5. Colonisation of virus-bearing aphides upon non-solanaceous hosts such as cabbage for periods varying from 24 hours to 7 days did not affect the subsequent infective power of such aphides.

6. *M. persicae*, when colonised upon plants affected with combinations of viruses of which leaf-roll was one constituent, transmitted only leaf-roll to healthy potatoes. The combinations used were leaf-roll and streak, and leaf-roll and mosaic. In the latter case the aphid was shown, by its infection of tobacco with a mottling disease, to be picking up both viruses of which only leaf-roll developed in the potato.

7. The incubation period of the leaf-roll virus in the plant averaged

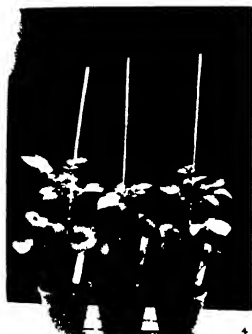
about 30 days under glasshouse conditions. In some cases the disease was found to develop in 18–20 days.

8. Seven varieties of potato have been infected with leaf-roll by means of *M. persicae*. There existed no apparent difference in degree of resistance to leaf-roll.

9. The leaf-roll virus can be disseminated by the feeding of *M. persicae*, either on the sprouts of the tuber, on the leaves and shoots of the growing plant, or on the stem alone.

#### REFERENCES.

- (1) ELZE, D. L. The Dissemination of Virus Diseases of the Potato by Insects. *Inst. voor Phytopath. Lab. voor Mycol. en Aardappelonderzoek. Meded.* xxxii, 1927.
- (2) KUNKEL, L. O. Studies on Aster Yellows. *American Journal of Botany*, xiii, No. 10, December 1926.
- (3) MCCLINTOCK, J. A. and SMITH, L. B. True Nature of Spinach Blight and the Relation of Insects to its Transmission. *Journ. Agric. Research*, xiv, July 1918, pp. 1–59.
- (4) MURPHY, P. A. and M'KAY, ROBERT. *Sci. Proc. Roy. Dublin Soc.* xviii, N.S., No. 14, p. 177.
- (5) SMITH, KENNETH M. Studies on Potato Virus Diseases. IV. Further Experiments with Potato Mosaic. *Ann. App. Biol.* xvi, No. 1, February 1929.
- (6) — A Comparative Study of the Feeding Methods of Certain Hemiptera and of the Resulting Effects upon the Plant Tissue, with Special Reference to the Potato Plant. *Ann. App. Biol.* xiii, No. 1, February 1926.
- (7) STOREY, H. H. Transmission Studies of Maize Streak Disease. *Ann. App. Biol.* xv, No. 1, February 1928, p. 15.















## EXPLANATION OF PLATES X—XII.

## PLATE X.

- Fig. 1. Arran Victory plant arising from sprouted half tuber infected by *M. persicae* late in the season of 1927, showing "primary" leaf-roll.
- Fig. 2. Progeny of the plant shown in Fig. 1 grown in 1928, exhibits marked secondary leaf-roll.
- Fig. 3. Healthy controls to plants shown in Figs. 4, 5, 6.
- Fig. 4. Three plants of Arran Victory, arising from sprouted half tubers infected by *M. persicae*, February 1928, showing "primary" leaf-roll.
- Figs. 5, 6. Two of the three plants shown in Fig. 4 photographed six weeks later, now showing "secondary" leaf-roll. The 24 plants, of which these are two, were as severely affected in the current season as the plant shown in Fig. 2 which was infected the previous year.

## PLATE XI.

- Figs. 1, 2, 3. President plants, showing advanced leaf-roll, arising from sprouted half tubers infected by *M. persicae*, current season infections. Note the stiff, upright habit characteristic of this variety.
- Fig. 4. Leaves of leaf-roll potato, var. President, showing the presence of pale yellow coloration at the leaf bases on the upper surface. This coloration is typical of leaf-roll in President. The lower surface of the leaves often exhibits a pink pigmentation. Compare Fig. 5.
- Fig. 5. Leaves of leaf-roll potato, var. Arran Victory, showing the brownish-black pigment at the leaf bases on the upper surface. This pigmentation is characteristic of leaf-roll in Arran Victory. Compare Fig. 4. (The leaves illustrated in these two figures were photographed between two glass plates to flatten out the rolling.)
- Fig. 6. Current season infection of Arran Victory with leaf-roll. This plant was one of a series infected by *M. persicae* feeding on the haulm, instead of the sprouted half tuber.

## PLATE XII.

- Fig. 1. Current season infection of Arran Victory with leaf-roll. The plant on the left was infected by virus-carrying aphides (*M. persicae*) after they had fed for 72 hours on cabbage, that on the right in the same way after 7 days on cabbage.
- Fig. 2. Leaf-roll streak Up-to-Date plant used in experimental infections. Note the rolling of the leaves and the killing of the shoots by streak (x).
- Fig. 3. Infection of different potato varieties with leaf-roll by *M. persicae*. Edzell Blue.
- Fig. 4. Infection of different potato varieties with leaf-roll by *M. persicae*. Great Scot.
- Fig. 5. Infection of different potato varieties with leaf-roll by *M. persicae*. King Edward.

Photographs by C. W. Williamson.

(Received October 9th, 1928.)

## INVESTIGATION OF HOP MOSAIC DISEASE IN THE FIELD

BY W. F. CHEAL.

*(Department of Plant Physiology and Pathology, Imperial  
College of Science and Technology, London.)*

(With 2 Text-figures.)

DURING the years 1923-5 field observations of Hop Mosaic were carried out to study more fully the symptoms of the disease, to follow more completely its spread under commercial conditions, and at the same time to determine how far the disease can be controlled by the prompt grubbing of diseased plants. These observations, made in a number of gardens in various parts of Kent, extended over the three years, and the following is a summary of some of the results that may be of interest on the practical side.

### INFECTIOUS NATURE OF THE DISEASE.

In the garden *B*, where the disease was rampant, a study of the position of diseased hills occurring during the three years, shows that although plants far removed from other diseased plants may develop the disease, yet in general the disease clearly spreads from areas which have been occupied by such plants.

The history of this garden was interesting. A small portion in the middle of the field of Tutsham plants had been planted with certain seedlings (*M.* 45, etc.) and the following year a big outbreak of Mosaic Disease occurred among the Tutshams surrounding the imported seedlings. The seedlings and the diseased plants were grubbed during the following winter (1922-23).

The position of those seedlings was the centre of the area which embraced nearly all the cases of Mosaic Disease found during the three years of observation (see Fig. 1, p. 231).

All the evidence collected shows that the disease was introduced to that garden by these seedling varieties which did not themselves show the disease (carriers).

As regards observations in the other gardens they, too, gave support to the view that the Mosaic Disease of hops is an infectious disease, as grafting experiments have since shown.

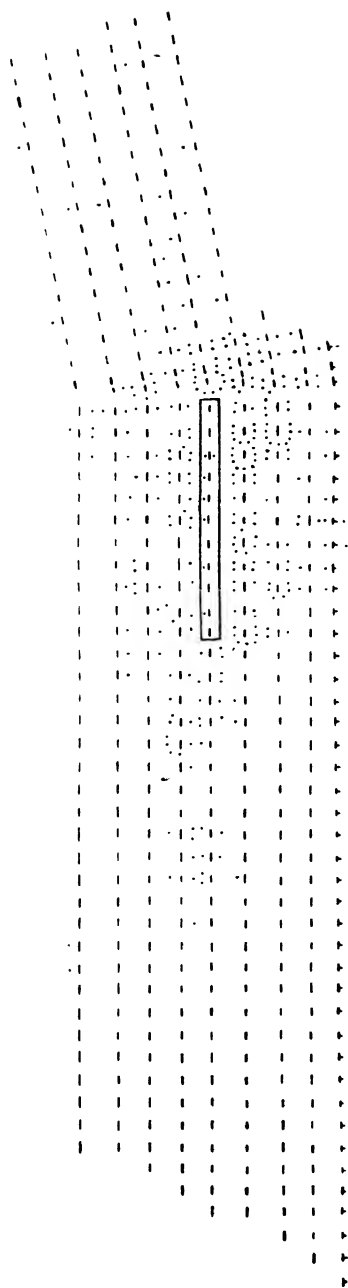


Fig. 1. Plan of garden B showing the position of hop plants developing Mosaic Disease during the years 1923-5. The diagram is approximately to scale. Each dot represents a single diseased plant. Each *short* line signifies 16 hop plants, and each *long* line 12 hop plants. The portion enclosed in the rectangle is that area in which certain seedlings, not Tutsham, were planted in 1921. One or more of these seedlings was a carrier of the disease. The other plants in this garden are all of the Tutsham variety.

## SYMPTOMS OF THE DISEASE.

The detection of the initial symptoms of Hop Mosaic presented considerable difficulty. Plants did not show symptoms until they attained the height of 3-4½ ft. Even diseased plants marked down and specially watched the following year did not exhibit symptoms until they reached that height. As a rule, the first indication of the disease was found in the



Fig. 2. Photograph of two shoots from the same plant (var. Tutsham). Left: the normal shoot. Right: deformed shoot from a diseased lateral.

leaves at the tip of the plant. A period of 5-7 days was quite sufficient for a plant hitherto normal to develop definite signs of bad Mosaic Disease.

Throughout the three years various types of mottling were encountered in all the gardens. They were classified and one special type is of importance. In 1923, two or three weeks before the picking, a big outbreak of this particular type appeared in all gardens under observation. The

mottling occurred, as a rule, on a few short laterals (usually no more than three) at the head of an otherwise perfectly normal plant. When these laterals bore hops, the cones were deformed and undersized (Fig. 2) and, in some cases, the mottled laterals died back at the tips.

Thirty-three cases of this mottling were found in one garden. The following year (1924) 21 were badly affected with Mosaic Disease; the remaining 12 were normal. In 1925 one of the 12 showed bad Mosaic Disease, while the 11 continued healthy in appearance.

In another garden, 30 cases of this same mottling were marked down for observation. In 1924, 20 developed definite Mosaic and were grubbed, 9 were normal, and 1 was suspect. The following year all 10 were normal.

The same type of mottling was found in 1924, and 10 cases found in one garden were watched. The following year 6 of them developed definite Mosaic Disease; the remaining 4 were normal.

These figures indicate that two-thirds of the plants showing this symptom one year will have Mosaic Disease the following year. The remaining third may be quite normal for the second and third years, or in a very few instances show definite Mosaic symptoms in the third year. The longest time any of these "mottled" plants may remain normal in appearance in succeeding years, whether any ultimately recover or whether they retain the latent form of the disease, has not yet been determined.

It should be stated that a large number of bad cases of Mosaic occurring in 1924 were perfectly healthy in 1923.

There is no doubt that this disease can be present in an apparently healthy plant of a susceptible variety, as the history of the following case shows.

1923. A one-year old plant.

June 8. Regarded as a suspect.

July 6-8. Definite *Mosaic Disease*.

Aug. 2-5. Improved considerably.

Aug. 30-Sept. 4-5. *Quite normal*. No mottling on the laterals and the cones perfect.

1924.

May 5-8. Normal (in any case too small for symptoms to appear).

May 15-18. Normal.

June 11-12. Suspect.

## 234 *Investigation of Hop Mosaic Disease in the Field*

1924.

July 25.                    *Definite Mosaic Disease.* It had a "grow away" tendency, but the disease had the upper hand. It bore a small crop of deformed cones associated with definite Mosaic mottling.

1925.                    *Definite Mosaic Disease.*

During conditions favourable for rapid growth diseased plants may produce growth free from disease symptoms, but they will not maintain the improvement after the conditions for rapid growth have passed<sup>1</sup>.

### EFFECT OF GRUBBING ON THE CONTROL OF THE DISEASE.

With our present knowledge of the symptoms of the disease, prompt grubbing carried out over a period of two years will not entirely stamp out the disease, but it will check the spread and, in some cases, reduce the amount of disease in the third year.

In the garden to which reference has already been made in regard to the introduction of the disease by certain special seedlings, grubbing appears to have done much good.

The figures are:

1923. 104 cases: grubbed, 74; the remaining 30 were some of those showing the special type of symptom referred to on p. 232.

1924. 260 cases (240 new): grubbed, 259 including 20 of the 30 left in from last year. One left by error.

1925. 18 cases (17 new): these included the one left in from 1924.

That the low figures for 1925 may be due to the season masking the disease does not seem likely, since a garden about 10 miles away was very badly attacked with Mosaic Disease that year. Furthermore, an inspection in 1926 showed that the improvement was maintained.

In another garden of half the area where prompt grubbing was not carried out the figures for the three years are:

1923. 37 cases: grubbed, 4; 33 left in (all those showing the new symptom).

1924. 222 cases (201 fresh): grubbed, 164, about six months after the crop was harvested.

<sup>1</sup> This may account for a belief that a dressing of nitrate of soda will cure the disease. No cases were observed to recover permanently, and no correlation between manuring and incidence of the disease could be established.

1925. 83 cases (about 30 new): these included about 50 of those left in from 1924.

Unfortunately a strict comparison of this small garden with garden *B* is not allowable, since in the small garden there is the possibility that part of the garden had been planted with stock infected with a latent form of the disease, and further, a badly infected garden adjoined it, although that garden was being rogued of the disease.

#### RECOMMENDATIONS.

The disease is infectious in the field and for that reason prompt grubbing is to be recommended.

Prompt grubbing carried out for a period of two years will not stamp out the disease but will hold it in check.

Where a grower decides to rogue his garden of diseased plants he should examine the plants at least twice in the season; firstly when the plants are about 4–6 ft. high (*i.e.* at the breast wire in the Butcher System of training), and a second time two or three weeks before the picking.

Growers should be extremely careful to avoid the importation of “carrier” plants into their gardens.

Growers should avoid taking sets from infected gardens, also in infected gardens the practice of filling up gaps with cuttings from neighbouring hills cannot be too strongly condemned, as the disease may be present in a plant which is perfectly normal in appearance.

*(Received August 19th, 1928.)*

# OBSERVATIONS OF THE *HELMINTHOSPORIUM* DISEASES OF CEREALS IN BRITAIN

## I. THE BEHAVIOUR OF *HELMINTHOSPORIUM GRAMINEUM* IN A COMMON BARLEY DISEASE

BY NOEL J. G. SMITH, PH.D. (Cantab.).

(*Rhodes University College, University of South Africa.*)

(With 3 Text-figures.)

### CONTENTS.

	PAGE
1. Introduction . . . . .	236
2. Methods of investigation . . . . .	239
3. The sources of the attack on the germinating grain . . . . .	240
4. The possibility of seedling-infection from the soil . . . . .	243
5. The fungus in the sheaths and leaves of the seedling . . . . .	244
6. The external manifestations of the disease and their interpretation (earlier stages) . . . . .	245
7. The stripe form . . . . .	247
8. The correlation and distribution of the stripes . . . . .	248
9. Escape of the later leaves . . . . .	249
10. The fungus in the stem and growing-point . . . . .	250
11. Infection of the stem . . . . .	250
12. The consequences of the infection of the growing-point . . . . .	253
13. The fungus and the young grain . . . . .	254
14. "Blindness" in leaf-striped barley . . . . .	255
15. The disease and the tillers . . . . .	256
16. The tillers which escape . . . . .	256
17. The time for observing the incidence in a crop . . . . .	257
18. Infection experiments . . . . .	257
19. Summary . . . . .	259
References . . . . .	259

### 1. INTRODUCTION.

THIS paper is an account of part of a re-survey of the *Helminthosporium* diseases of British cereals. The work was commenced in 1922, at the suggestion of Mr F. T. Brooks, and was mainly carried out at the Botany



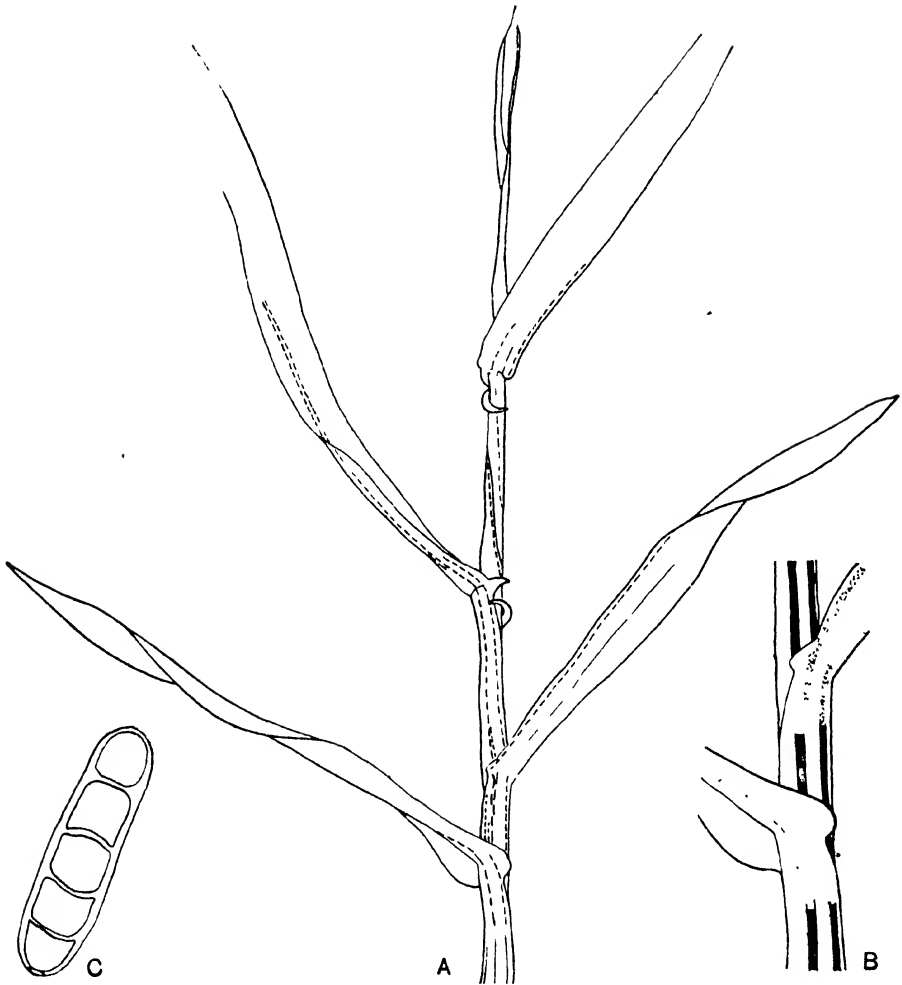


Fig. 1. (Drawn from a typical specimen.) (A) shows the upper part of a barley plant. The dotted lines represent the stripes of diseased tissue. Attention is particularly called to the tightly-wrapped sheaths. For part of the plant these are shown on a larger scale in (B).

In (B) the stripes, on the tightly-wrapped portions of the sheaths, are represented by heavy shading. Note how near the heavily-shaded portions of the second leaf are to being in line with the similarly shaded parts belonging to the first leaf.

(C) is a typical conidium of *H. gramineum*.

School, Cambridge. For Mr Brooks' constant helpfulness during the investigation I have here to record my thanks. The disease which is particularly dealt with in this paper is commonly known as Leaf-stripe Disease of barley. Fig. 1 will recall the characteristic form of damage, and the equally characteristic appearance of the commonest spore-form of the fungus.

The most convenient starting-point is formed by the work of F. Kolpin Ravn (7, 8). His work at once brought together the scattered and highly contradictory evidence, which had accumulated prior to 1900, concerning the *Helminthosporium* diseases of cereals, and compacted this by new discoveries. When the existence of two separate diseases of barley and one of oats caused by fungi of this genus had been made clear by Ravn's papers, the work of many authors made it apparent that the distribution of the diseases was very wide, and that their economic importance made them worth combating. Bibliographical references to these records of the distribution of the diseases, and of control measures which have been successful against them, are not given, the information being available elsewhere, notably in Drechsler's<sup>(1)</sup> paper.

When the conclusions of one worker, in this case Ravn, who of necessity dealt only with a section of one country, are utilised as a foundation for world-wide records, that foundation, naturally, is very severely tested. Conditions are rendered worse when the additions are made indiscriminately, resting completely on parts of the foundation which Ravn himself did not guarantee, or on some part which has been subjected to an ill-judged modification, or on some part which was guaranteed but was in reality inaccurate. In short, the mass of data concerning these diseases which has accumulated during this century is quite as formidable, in its confusion and insecurity, as the mass which confronted Ravn at the close of last century.

The two different diseases of barley which Ravn distinguished are (1) Leaf-stripe Disease, caused by *H. gramineum* Rabh., and (2) Net Blotch Disease, caused by *H. teres* Sacc. Among the important characteristics of Leaf-stripe Disease, which are not shown by Net Blotch Disease, Ravn pointed out that when the disease appears on one leaf of a plant then all the later leaves will show the disease, the stem and ear also becoming affected. Ravn also pointed out that infection of the seed is the only mode of initiating Leaf-stripe Disease in a plant.

As regards the host-parasite relation which is reflected in the symptoms, and in the mode of spread of the diseases, he concluded that *H. gramineum*, like certain of the Smut fungi, inhabits the growing-point of the

host plant, and thence spreads to each young part of the plant, during the formation of the part. Drawing a contrast he pointed out that *H. teres* mainly spreads, like the Rusts (Uredineae), by wind-borne spores.

Certain adjustments of Ravn's point of view have been suggested in recent papers, *e.g.* by Vogt<sup>(13)</sup> and by the present writer<sup>(10)</sup>. References to these and others will be made in the appropriate later paragraphs. Several papers which are of considerable indirect interest, as they deal with the validity of various characters for distinguishing *H. gramineum* from other species, are not mentioned here at all, inasmuch as the British *Helminthosporium* diseases of cereals must be compared with one another from several points of view in a later paper.

In the present paper the host-parasite relationship in Leaf-stripe Disease alone is dealt with. This is the first full presentation of facts which show that the parasitism of *H. gramineum* is fundamentally different in type from what has previously been supposed. It does not lead a Smut-like life, but a life which is interesting in its own way.

It has already been noted that Ravn, following up an earlier suggestion of Rostrup<sup>(9)</sup>, came to the conclusion that the mycelium of *H. gramineum* inhabits the growing-point of infected plants, and from there spreads to all the young tissues as they are formed. Of all his conclusions this would seem to be the one most requiring re-investigation. For even a casual glance at diseased plants raises doubts. If the mycelium causes this sickening and death of cells, wherever it penetrates in the leaves, sheaths, etc., will it not then also kill invaded growing-point tissues? The appearance of plants dead or dying from the disease certainly suggests that it sometimes does. Or, why does the fungus spread in the leaves only in stripes, and stripes which, as will be seen later, are rather peculiarly arranged, if all parts have an equal chance of infection? These are only two of the most obvious doubts which present themselves. One also felt that the Smut analogy had led Ravn into an inadequate perception of the seed-borne sources of the disease, but these sources are discussed fully in some later paragraphs.

## 2. METHODS OF INVESTIGATION.

Though field observations yield valuable evidence as to the host-parasite relationship, the main basis for its understanding must be supplied by microtome sections. No complex *résumé* of methods experimented with is necessary. It must suffice to say that satisfactory sections were obtained when ordinary fixatives (*e.g.* Chrom-Acetic) were used, and ordinary stains (*e.g.* Heidenhain's Haematoxylin, with counter

stains such as Congo Red). Many of the main facts can also be shown up by the use of Lacto-phenol and Cotton blue.

Cases of the three *Helminthosporium* diseases which affect barley in Britain, viz. *H. gramineum*, *H. teres* and *H. sativum*, were under observation during the present investigation. Parallel studies of the three organisms in pure culture and in infection experiments were also carried out. This gives a great measure of security against confusion regarding the identity of species.

As it is much more easy to differentiate *H. teres* and *H. gramineum* by their mode of growth in culture than by the symptoms which they cause in affected plants, it was essential to establish the main facts from plants grown from clean seed and artificially inoculated with the respective fungi. When naturally-infected plants had to be used as a source of material the identity of the *Helminthosporium* and its connection with the hyphae observed in sections was made certain. When the surface of infected tissues is sterilised, the internal mycelium readily grows out into culture media. After considerable knowledge of the conidia, as produced under various conditions, had been gained they also became a useful means of identification.

### 3. THE SOURCES OF THE ATTACK ON THE GERMINATING GRAIN.

The sources of attack carried in or on the grain are (1) conidia, (2) mycelium, and (3) sclerotia, which may develop into perithecia.

(1) *Conidia* (or conidiophores) may be present on the grain whether it was produced on a diseased or on a healthy parent. This source was considered by Ravn(8) to be mainly responsible for Leaf-stripe Disease. Other sources will be considered later, but these, though more effective in the production of diseased plants, are less widespread. Conidia are produced so abundantly that even a few diseased plants might well contaminate a great proportion of a crop, during harvesting, stackbuilding or threshing operations. There is fortunately, however, little certainty of infection from this source. Ravn obtained ten positive results from 200–250 inoculated grains. The chance of infection from natural contamination with isolated spores must be considerably less than this.

In the closely adherent chaffs of the barley there is normally only one gap through which the fungal germ-tubes may readily enter, or through which the young barley shoot may push its way on germination. This is the aperture left by the incomplete overlapping of the pale by the flowering-glume, at the apex of the grain where the base of the awn is.

In this vicinity conidia may obtain lodgment, and, putting out germ-tubes when the seed is sown, may reach the coleoptile of the emerging shoot. Elsewhere the chaffs give less opportunity for the lodgment of spores and form a barrier which must be circumvented by the germ-tubes. Zade<sup>(14)</sup> similarly dismisses as slight the chance of effective penetration by spores of *Ustilago avenae* present on the outside of oat grains.

(2) *Mycelium in the Chaffs and Pericarp.* When the fungus is considered in relation to the flowering stage of the barley it will be shown that this awn-end of the chaffs is the part which is most likely to contain mycelium as well as conidia. This is because the presence of such mycelium in the grain-coats results either (1) from the ear of a diseased plant brushing against the fungus-containing sheath (of the last leaf) during its upward passage; or (2) from a grain of a healthy plant being infected, while the chaffs were still soft, by a spore from a neighbouring diseased plant.

That mycelium could be present in the chaffs of germinable grains from the first source did not appeal to Ravn as being other than a rare possibility. He believed that plants with Leaf-stripe, as with Smut, rarely produce germinable grains. From the attitude which is taken up in the present paper it will be apparent, especially later, that the grains which are produced on diseased plants are to be considered as liable to show all degrees of infection, those showing slight, moderate, or even fairly severe infection being germinable. Thus the importance of mycelium from the first source has now to be considered greater.

That mycelium could be present in the chaffs and kernel of germinable grains from the *second* source was mentioned by Ravn<sup>(7)</sup>. Vogt<sup>(13)</sup> further emphasised the importance of this mycelium, but it is by no means certain that he was dealing with *H. gramineum*. Ravn did not describe the nature and distribution of this mycelium. If he investigated it at all, his attitude towards it must have been very different from mine. Observations of the mycelium in the chaffs in some cases, in the kernel in others, and in the embryo in still other cases, he must simply have considered as observations of the progress of the fungus nearer and nearer to its proper goal, inasmuch as he considered that the infection of the embryo is the first step in the initiation of Leaf-stripe disease. I consider, on the other hand, as will shortly be seen, that an embryo which has mycelium in its near neighbourhood is far from likely to produce a typical Leaf-stripe plant, and is, in fact, likely to produce no plant at all.

From either source, then, the quantity of mycelium which is able to establish itself in the chaffs, and in the inner parts of the grain, is liable to vary greatly. A very small quantity, causing only very slight discoloration of the chaff, is sufficient as a source of the disease. At the other extreme, when the endosperm, and even the embryo, is invaded, the quantity is so much more than sufficient that the plant is overwhelmed either before germination or very shortly after. While the lightly infected grains must be the source of a far greater number of Leaf-striped plants, yet these cases which are but one stage better than ungerminable seed are also illuminating as to the nature of the fungus.

The region in which the *Helminthosporium* hyphae find a passage from the outer seed-layers to the endosperm is at the basal end of the furrow of the grain where the end of the aleurone layer abuts on the scutellum. Elsewhere the crushed remains of the seedcoat, cross-cells and tube-cells usually form an effective barrier to the penetration of the aleurone layer. Its slow progress in closely packed tissues is a characteristic of *Helminthosporium*, exemplified in the behaviour of mycelium penetrating the embryo through the scutellum. Closely packed cells have immediately to be met, and the fungus from this source is often left impotent for a considerable time, only assisting in the general lowering of the vitality of the plant. This is more effectively produced by the fungus from other sources, which have now to be considered.

Mycelium in the chaffs and pericarp, we have seen, is inevitably present when the endosperm is invaded, and may be present even when the endosperm contains no fungus. This mycelium in the outer seed layers can remain viable for at least two years and under the damp conditions of germination produces conidia and fresh hyphae. These are the effective agents in penetrating the primary sheaths, *i.e.* the coleorhiza (root-sheath) and the coleoptile (plumule sheath). Penetration of the coleorhiza, or of the rootlets which arise from it, results in rotting, stunting, or, at the least, in impaired efficiency of the root system, which is a contributory cause of sickliness in the plant.

Penetration of the coleoptile, on the other hand, is the first step towards the production of Leaf-stripe. The first opportunity for such penetration occurs when the shoot, covered by the coleoptile, commences to push its way between the inner surface of the chaff and the outer surface of the kernel. On one or both of these surfaces fresh fungal hyphae may be developing. The young shoot is further menaced at its point of emergence from the chaffs, since here may be met hyphae spreading on the outer surface of the chaffs.

The form in which this important chaff mycelium overwinters is interesting. Much of the mycelium becomes changed into a very resistant form, with thick walls and the cells short, often swollen. This resistant mycelium would deserve more detailed consideration were it not that Vogt<sup>(13)</sup> has described, at considerable length, a similar mycelium in chaffs affected by the species of *Helminthosporium* with which he dealt. The same type of resting mycelium occurs in cultures when the medium is drying up, and is also known in other fungi (particularly those tending to produce sclerotia) when the environment is becoming unfavourable for further vegetative growth.

(3) *Sclerotia and Perithecia*. It is probable that all the spherical or pyriform sclerotia which are formed on the surface of grain tissues, or within them, can be regarded as potential perithecia. On the other hand, it is doubtful whether ascospores are formed often enough, or early enough, to be a considerable source of danger to the young plants. In this very resistant form, however, the fungus is likely to be more difficult to kill than the embryo itself, *e.g.* by seed-disinfectants, and it is certainly capable of producing numerous conidia and fresh hyphae under the moist conditions of germination. This "resting-stage" is thus of practical importance.

In a paper published during the course of this work, van Poeteren<sup>(5)</sup> describes the production of sclerotia of "*Pleospora trichostoma*" on the outside of diseased grains (from Leaf-stripe plants) when these have been kept for three days in a damp atmosphere. The only sclerotia which I have found definitely associated with the *Helminthosporium*, under these conditions, have been of the rather unorganised type which Noack<sup>(4)</sup> terms "nests of mycelium." There are undoubtedly sclerotia present in or on some diseased grains throughout the winter. Early stages of ascus-formation which were seen in some of these will be described later.

#### 4. THE POSSIBILITY OF SEEDLING-INFECTION FROM THE SOIL.

It is necessary to consider the soil as a possible source of fresh outbreaks, but that consideration can be brief. The fungus undoubtedly reaches the soil in as many forms as it reaches the chaffs. There are several viable forms present in ground which has been recently cleared of a diseased crop, and the viability of the sclerotia at least persists for some considerable time. Yet these viable forms, by the ploughing activities of the farmer and the competing activities of micro-organisms will be placed for the most part in a position unfavourable for attack on a new crop. Even if the fungus in some viable form were favourably placed, *e.g.* very

near a germinating seed, still it would not have more chance of initiating Leaf-stripe disease than conidia on the outer parts of the grain have. The chance of infection from these conidia has already been dismissed as slight.

It is an undoubted fact, as previous authors have mentioned, that the distribution of Leaf-stripe plants over a crop is sporadic, not in marked patches. This suggests, among other things, that Leaf-stripe is not a soil-borne disease. One agrees with Drechsler<sup>(1)</sup> that Johnson<sup>(3)</sup>, who greatly emphasised the capacity of *H. gramineum* to infect from the soil, was mistaken in his identification.

#### 5. THE FUNGUS IN THE SHEATHS AND LEAVES OF THE SEEDLING.

*Penetration.* From one or other of these sources, then, mycelium comes into contact with the coleoptile. When the mycelium has grown to some length, passing along the outer surface of this sheath, appressoria are formed, and a narrow "penetration hypha" pierces the host epidermis. The subsequent course of the penetrating hypha is, in my experience, between epidermal cells. Noack<sup>(4)</sup>, Ravn<sup>(7)</sup> and Stevens<sup>(11)</sup> record similar observations with kindred species of fungi and host-plants. Some observations show that the first attacked cell is invaded by mycelium, but thereafter the progress of the hyphae is fundamentally intercellular; the cells of the host are not invaded till they are in a very unhealthy condition. Such confirmation of Ravn's conclusion that the progress of this fungus is fundamentally intercellular was suggested as necessary by Stevens<sup>(12)</sup>, when he described the very different behaviour of *H. sativum*, an intracellular parasite.

*The Fungus in the Coleoptile.* The coleoptile is mainly composed of thin-walled cells, vascular bundles being present to the number of only two. The proportion of tissue easily traversed by the fungus is thus greater than in a leaf, and the result is more speedy invasion and a diseased area not strictly delimited as a stripe. Owing to the arrangement of tissues, however, the progress of hyphae in the cell walls which are parallel to the long axis of the shoot is uninterrupted, while progress towards the inner surface of the coleoptile must be effected by a zig-zag course. As would be expected from this, the fungus makes more progress upwards and downwards, from the point of penetration, than radially. The time elapsing before the inner surface of the coleoptile, which is in contact with the outer surface of the first leaf, is reached will vary according to whether conditions are in favour of host or parasite, *e.g.* in the number of hyphae which have effectively gained entry.



In general it is found that hyphae have reached the epidermis of the coleoptile, have broken through it, and are present on the moist inner surface, while the first leaf is still growing up in contact with this surface. Penetration of the leaf (or its sheath part) thus occurs on its outer surface either before or soon after its emergence, just as did penetration of the coleoptile from the adjacent chaff or grain tissues.

The other possible mode of attack, where the hyphae invade the leaf from the node which produces it, must depend on the possibility of the mycelium travelling down the coleoptile to its base, then up the stem internode to the node of the first leaf. The amount and nature of the tissues that would have to be traversed either renders the threat from this source abortive or results in hyphae gradually making progress in the meristematic regions. As in the case of mycelium penetrating these regions from the scutellum, these hyphae cannot be regarded as effective in producing Leaf-stripe in new leaves, but can be regarded as contributing in a greater or less degree to sickness or death of the plant.

When the first leaf passes up through the fungus-containing coleoptile the possible results for the leaf may be summarised as (1) death (meristem invaded), (2) Leaf-stripe (lateral invasion), (3) escape. The second leaf wrapped up within the first has in turn to face the same possibilities, and so on for all leaves, sheaths, and finally the young ear. Death may come with varying speed, Leaf-stripe may be developed to a varying degree, and escape may be temporary or permanent.

#### 6. THE EXTERNAL MANIFESTATIONS OF THE DISEASE AND THEIR INTERPRETATION (Earlier Stages).

A general idea of the potentialities of the fungus, working within the host, has been gained by considering in detail the processes which result in the infection of the earliest invaded parts. As the plant grows older the results of these activities become accessible for macroscopic examination, and this large-scale study yields evidence corroborating that of the microtome sections.

*Changes in the Leaf.* Typically the young leaf on emerging, or soon after, shows the presence of the fungus in one or more stripes. These are, at first, only apparent locally, and distinguishable mainly because of their translucency as compared with the neighbouring healthy tissue. This was noted by Ravn (8), though perhaps with too definite an emphasis on the earliness of the appearance of this stage. The time required for a sufficient massing of the fungus to produce this macroscopically evident system, indeed, varies greatly. After this, and before the often-described

stage of the brown "mummified" stripe is reached, there is a definite stage when the stripes are distinguishable only by their loss of green colour. According to Ravn (7) the internal disorganisation, which is associated with this loss of colour, is confined to the mesophyll. "The chloroplasts lose their colour to a greater or less extent, and may be altogether destroyed. . . . Two types of hypha are present in the mesophyll, (1) hyphae which are quite straight for long distances, and (2) branches from these long hyphae, which, with a more or less curved course, distribute themselves, mainly in the transverse direction, throughout the mesophyll." The full significance of the abundance of the long hyphae is considered later.

It is worth while to emphasise further a point which has already been implied in the summary of the microscopic evidence, which was given at the end of the last section. The point is that even in a leaf which is showing only the "pale stripe" stage, the fungus is in a position to penetrate a new leaf.

From the quotation which has just been given, it does not seem that Ravn would have considered such a contention unreasonable. Not having looked at the distribution of the mycelium from this point of view, he limits himself too much to a description of the mesophyll damage. From the main mass of hyphae in the mesophyll a few, at least, of the transverse branches do pass between the cells of the inner epidermis, and so come into contact with the surface of the next leaf. The fact that, when a "pale-striped" leaf is kept in a damp atmosphere, mycelium grows out from the stripe into the air, often in quantity, may also be commented upon.

The mere starvation of the affected tissues, which must result from the presence of so many fungal hyphae, could probably account for this loss of colour, just as starvation, in the absence of any pathogen, can cause yellowing of leaves in general<sup>1</sup>. Though Ravn uses the rather more non-committal terms "afblegningstadium" (Dan.) or "Verblassungsstadium," yet one feels that the term "yellowing," with reference to this phase, is not inapplicable. It has been my experience that the "yellow-stripe" stage, which, quite often, persists as the only sign of the disease on plants showing several leaves, has proved puzzling to many observers. This is not surprising, in view of the scant notice which it has received in most published accounts of the disease.

The time of appearance of the first brown areas, within the pale stripe,

<sup>1</sup> I am indebted to Dr H. Godwin for suggesting this as a corollary on his own work on the physiological aspects of the yellowing of leaves.

thus varies and the spread of the "mummification" is also somewhat erratic. Typically, however, a diseased leaf shows, within a few weeks of its unfolding, the brown strip extending for practically the whole length of the leaf. A clear idea of the final state of the stripe—brown, shrivelled and bearing conidia—is given by Ravn's account.

#### 7. THE STRIPE FORM.

An investigation of the reasons for the disease appearing in this stripe form yields interesting results, and comparison with some Smut-diseases, which also produce lesions of the stripe form, on leaf and sheath tissue, shows that the distribution of the stripes, in Leaf-stripe disease, is dependent on factors different from any which have been noted as operating in these other diseases.

One factor operating in this disease, in common with most other stripe-forming diseases of the Gramineae, is the *mechanical resistance* offered by the strengthening tissues, which are present in and around the vascular bundles. It is clear that these bands, arranged lengthwise in the leaf, form a bounding line which is only slowly overpassed by the mycelium. The vascular bundles of very young leaves form a less effective barrier, and also bundles which have been involved in the general disorganisation of tissues in a "mummified" stripe.

It has been seen that in the coleoptile the influence of such tissue is little felt, but that the arrangement of cells tends to promote longitudinal spreading of lesions. (Macroscopically this is not usually very clear, as the "mummification" is only contributory to the natural collapse of the sheath quite early in the life of the plant.) Thus, when hyphae break through the inner epidermis and come into contact with the first leaf, *penetrations are likely to occur on a longitudinal strip or strips*, corresponding to those on the coleoptile. The upward growth of the leaf will also *brush a vertical strip* against the externally applied mycelium. The inrolled part of the leaf will escape penetration at this time, as will a greater or less proportion of the exposed surface, owing to the variable amount of mycelium which will have traversed the coleoptile.

The *sheath part* of the first leaf will be infected, just as is the blade part. This sheath part in the first few leaves, the "basal rosette," is much less strengthened than in the later leaves, a point which is in favour of the fungus. Though the sheath-stripe may remain in the yellow stage for a much longer time than the blade-stripe, it is still at that stage capable of infecting the young second leaf which passes up inside it. Its more lasting contact with the coleoptile renders it more liable than the leaf

blade to infection, and its closer relation to the second leaf makes it more potent in infecting. The inevitability of the presence of this sheath-stripe has not been appreciated, the literature giving rather the impression that it is a phenomenon that may or may not occur.

#### 8. THE CORRELATION AND DISTRIBUTION OF THE STRIPES.

The stripe on the second leaf would be expected to occur in the region of that leaf which was in contact with a stripe area on the first leaf. In the absence of complicating factors, some of which are noted below, this correlation is clearly seen, as in the plant figured (Fig. 1). It is more obvious in the cylindrical sheath parts of the successive leaves of an elongated shoot, since the arrangement of the still folded parts is nearer to that in a drawn-out telescope.

On the unfolding of the leaves, especially the later ones, it is commonly noticed that the stripe on the young leaf is broader than that on the preceding one. [In Raven's (7 and 8) coloured plate of the three uppermost leaves of a diseased shoot there are indications of this.]

The mycelium from the older sheath has been able to spread, either (1) before infecting the young leaf, *i.e.* on the surface of contact between the two leaves, or (2) after invasion of the young leaf has occurred. Lateral spreading during the second period will only be considerable if the leaf is penetrated while it is still very young. This will be realised from what has already been mentioned concerning the confinement of spread, on the part of the newly established fungus, to the line of least resistance, and concerning the vascular barriers which early become effective.

With reference to the question of spread on the surface of contact between the leaves, it is of interest that Raunkiaer (6) found that the narrow space between the sheath and haulm (of grasses) was constantly moist. He believed that the ligule assisted in the maintenance of this condition. The presence of moisture on the inner surfaces of sheaths, the outer surfaces of which are exposed to aerial drying, will render circumstances more favourable for the emergence of hyphae on the inner side of the sheaths, and especially for the spread of hyphae on this surface. Experience gives one the impression that such spread is mainly responsible for the successively broader stripes in the leaves and sheaths, passing from the lower to the upper.

Among the cases where the width and number of stripes increases as successive leaves are unfolded, there are some in which diseased tissue is apparently absent in the first leaf or leaves, in the first and second for example, throughout their whole life, yet a narrow stripe appears in the

third leaf, and the disease gains an increasing hold on all the later leaves. These cases are of interest since they suggest, at first sight, that there is some justification for the claim, made *e.g.* by Drechsler<sup>(1)</sup>, that this fungus, like the Smuts, can be present in the growing-point of the plant, even when no outward symptoms are visible. In all cases of this type which I have examined, however, mycelium has been found in the sheath portions of the earliest leaves. Mycelium in this situation, it has been noted, is dangerous though not conspicuous, and can be present without the necessity of mycelium being present in the blade. One must agree with Drechsler that in no case is the appearance of typical symptoms in the later leaves, of an apparently healthy plant, the result of secondary infections.

The observations recorded in the foregoing paragraphs all point to one conclusion, in addition to those already mentioned, viz. that the adult leaf is not a favourable environment for the spread of the fungus. Both as regards penetration from the outside (secondary infections) and as regards the spread of internally-present mycelium, the adult tissue is clearly much more resistant than the tissues in which the fungus first establishes itself.

One is rather puzzled by Ravn's observation that "little by little the mummification spreads over the whole leaf-surface, the green colour of which can only be observed as narrow stripes along the edge" (7). It is clear that, if this were constantly to occur with every leaf, one could hardly observe many of those cases which have been noted in recent paragraphs, *e.g.* the much narrower stripes on the much older leaves. It has rather been my experience that mummification, little by little, over-spreads that portion of the leaf-surface which has shown the pale-stripe stage.

The only marked extension of the diseased tissue, in an adult leaf, takes place at the base of the blade. Extension there results either from the spread into the blade of a "late" sheath-stripe, or from the germination of conidia in the basin occurring at the base of the blade, where the auricles clasp the haulm. Yet, even from these sources, the progress of the *Helminthosporium* mycelium upwards, into the drier and tougher parts of the leaf, is slow.

### 9. ESCAPE OF THE LATER LEAVES.

Plants which continue to produce a series of striped leaves (and, ultimately, a more or less diseased ear) have mainly been kept in view up to the present, since they provide the fullest demonstration of the working

## 250 *The Helminthosporium diseases of Cereals in Britain*

of the fungus in the host. Departures from this type, however, frequently occur, and these, because of their interest, will have to be referred to from time to time.

Plants which are affected with this disease may cease from producing the normal series of striped leaves, from either of two causes. Firstly, death may intervene at any stage of the life-history of the host, from causes already described. Or secondly, the later leaves of the plant may escape.

Such cases of permanent escape are fairly frequent. For instance, in an experiment which involved a close watch on 113 plants, which showed infection of the lower leaves, 26 plants showed escape of the upper leaves and ear. In such cases the fungus had a bad start, and has ever since failed to "live up to the time-schedule" necessary for the production of a fully diseased plant. It will be realised that, on occasion, conditions may favour the overcoming of this initial lag. More often, however, the lag is accentuated.

### 10. THE FUNGUS IN THE STEM AND GROWING-POINT.

When one looks, then, at the leaf symptoms of various plants, one obtains interesting evidence. When one examines the mature stems and the exposed ears of diseased plants also one can deduce with fair safety the sources from which the stem internodes and the ears become infected. This is true though the actual infection takes place before ever the internodes and ear become exposed to view.

Thus for giving the simplest picture of the course of the disease it would scarcely be necessary to discuss what happens while the stem and the young ear are still hidden within sheathing leaves, although one is compelled to discuss these happenings to some extent, for the sake of thoroughness, but, still more, because Ravn particularly chose, as his most striking evidence, cases at this stage of development.

### 11. INFECTION OF THE STEM.

Any leaf (*a*) arising at node (*A*) has the lowest part of its sheath in contact with the internode *AB*, and the remaining part in contact with the sheath part of the leaf which arises from the succeeding node (*B*). In a diseased plant the leaf arising at *A* will become infected from the one which ensheaths it, and in due course its surface which is in contact with the above-named parts of the stem and sheath will transmit the fungus to them. From this source, then, each stem internode when in a more or

less extended and hardened condition, is penetrated from the same source and at the same time as the leaf arising immediately above it. Mycelium from this source will not reach node *B* before it has reached the leaf (*b*) which arises from it, *i.e.* it will not be successful in initiating infection in the leaf through the (nodal) growing-point which gives rise to the leaf.

Internode after internode is thus infected from the "external application" of hyphae to it. After the "application" not unnaturally the hyphae spread in the stem, finding the epidermis and the pith in particular fairly easy tissues to which to travel. Mycelium thus reaches the pith of the successive internodes as a consequence of a vigorous mycelium being present in the leaves. It is one of the essential differences between Ravn's view and mine that Ravn considered that the mycelium reaches the leaves as a consequence of the presence of mycelium in the pith.

On any theory the first pith mycelium must be considered to have reached the pith from some form of "external application" (*e.g.* conidia germinating on the seed). Thereafter, on Ravn's view, the pith mycelium speeds on as the prime mover, leaving in its wake diseased leaves. On my view infection of leaves and stem-parts by "external application" continues, the diseased leaves leaving in their wake diseased internodes. Enough has been said to show how the facts concerning the distribution, for instance, of the stripes on the leaves fit my view. I cannot, in view of all the facts, regard the pith mycelium as more than a somewhat laggard co-operator which arrives to rot<sup>1</sup>, to a small extent, the core of structures which are much more stoutly beset in their peripheral parts. In that case, one might almost say, there is being laid down a slow-spreading, tenuous, and by no means continuous stripe or stripes of diseased tissue. The stripe is confined to the core since the vascular bundles and some of the cortical tissue act as barriers. [It is significant that Ravn never found mycelium in the vascular bundles or cortex (*(7)*, p. 27).] The slow spread and the by no means uninterrupted course of the stripe is the result of the closely packed tissues which have to be negotiated, particularly at the nodes. Even in very young nodal regions the hyphae are retarded.

The above mentioned conclusions as to the laggard nature of the pith mycelium are the result of much careful consideration of what was revealed by many microtome sections. To quote in more detail the data which these provided would be to make too much of the matter.

It would have been, perhaps, most satisfactory to compare the relative potency of the pith mycelium and the mycelium derived from

<sup>1</sup> Rot is perhaps too metaphorical an expression, but certainly, as Ravn pointed out, the walls of the cells surrounding the hyphae turn brown, the cells dying.

“external application,” utilising the data which Ravn gives. Unfortunately he gives insufficient data. Of the two sets of mycelium whose

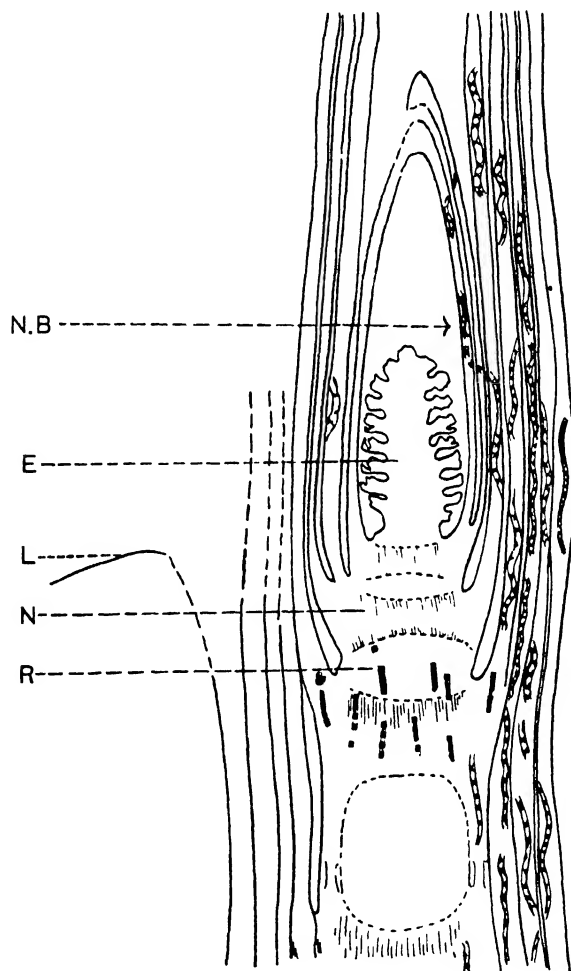


Fig. 2. Median longitudinal section of part of a young barley plant. The growing region of the stem is shown, enclosed in the sheath parts of leaves. The blade part of only one leaf (*L*) comes into the figure. The nodal regions of the stem are indicated by light shading, as at *N*. For further explanation see text.

position one would like to compare, he only shows in full the position of one, the pith mycelium. Therefore the comparison cannot be made.

This part of the work is illustrated in Fig. 2. The figure, built up from several sets of microtome sections represents a section of part of a barley



seedling. The four uppermost internodes of the stem are shown, together with the rudiments of the ear (*E*) which is forming at the stem apex. Such a young stem would, in nature, be hidden from view within a tube formed of the sheathing bases of leaves. The leaves involved would comprise both the leaves which arise from the four nodes included in the diagram and other leaves from lower nodes. These leaf-sheaths are indicated rather diagrammatically. The blade parts of one leaf (*L*) also come into the picture. The leaf parts which are infected by hyphae are traversed in the diagram by snaky lines, inserted for the most part only in the right-hand side of the figure. Mycelium within the stem is also present. The position of this is carefully marked in the region (near *R*) which is the uppermost limit of its spread. In that region it is represented by straight dotted lines.

A figure which Ravn gives, both in (7) and (8), shows that when he found a quantity of mycelium within the stem, exactly such as is indicated at the level of the arrow (*R*) he was convinced that he had found the mycelium which, after the fashion of Smut mycelia, infects all the young parts. How, in actual fact, the leaf mycelium is in a position to anticipate it in the infection of new parts is shown particularly in the region of the arrow (*NB*). The young ear is going to receive an "external application" of hyphae, from the uppermost leaf, long before the mycelium in the interior of the stem has worked its way up the rachis and commenced to damage the ear from its core.

It will be realised that, when the hyphae in all parts of the plant have made rather more progress than in the case illustrated, weighing of the relative importance of the two sources of mycelium, central and peripheral, becomes difficult. The difficulty is the same as that which one encounters when one examines, with the naked eye, a barley plant every leaf, every stem part and every grain of which gives evidence of the presence of the fungus. One cannot say without knowledge of the history of the plant whether the stem was infected from the diseased leaves which ensheath it, or whether the leaves were infected from the diseased stem which produced them. These wholly diseased plants, like Ravn's figure, represent final phases, which do not necessarily bear clear evidence as to their previous history.

## 12. THE CONSEQUENCES OF THE INFECTION OF THE GROWING-POINT.

It has been shown, then, that first the leaves are infected, that from them the stem parts are infected in ascending order, and that, from both leaves and stem, hyphae converge on the growing-point. In other

words the whole order in which parts are infected is against the Smut analogy.

It has been stated also that the consequences of the infection of meristematic tissues is also against the Smut analogy, inasmuch as it has been stated that death (coming quickly or slowly) is the fate of leaves or whole seedlings when the meristem, which should undistractedly continue tissue formation, is laid under toll by fungal hyphae. It was stated that this sequence of events in the case of leaves or seedlings was deduced from microtome sections. It is of further advantage that this evidence should be confirmed when the activities of the fungus and their effects on the host tissues are alike demonstrable on a larger scale.

In viewing the large-scale activities of the fungus in the leaf the reader must have realised more and more that this is not a fungus which is likely to leave unharmed any tender tissues among which it may be dwelling. Even more pertinent evidence is provided, however, when the last internode, elongating at the time of the "earing" of the barley, becomes visible, to the naked eye. That this last internode, which ought to be long and straight, is in a diseased plant more or less stunted, and often so weak as to be cast into corkscrew coils, is well-known. There is good evidence to show that this distortion is the result of the fungus being present in the tissues of these internodes, the bulk of the damage having been done when they were young and tender. Some good evidence is afforded by statements made by Ravn. In the month of May he found that the position which the hyphae occupy in relation to the uppermost internodes was that represented by the position (*R*) in my figure (Fig. 2). In rather older stem-apices he found that mycelium had progressed as far as the uppermost internode. At the end of June he found that a great stunting of growth began. It is rather surprising that, in the light of these happenings, he did not interpret the fungus as being more of a disturber, and less like a symbiont.

### 13. THE FUNGUS AND THE YOUNG GRAIN.

The kind of menace which threatens a young ear has been described and shown (in the case of a very young ear) in Fig. 2. The number of the menacing hyphae, of course, varies, as does the degree of their success. Accordingly, for the ear, as for other parts, the three possible fates are (1) escape, (2) death, or (3) the reception of a greater or lesser quantity of mycelium. Ears meeting the third fate are the ones which interest us here.

When the state of the mature grains of such ears was being discussed

(in connection with the forms in which the fungus is present on the seed as sown) it was stated that the headquarters of the mycelium in the chaffs is at the awn-end. The reason for this was also briefly indicated, namely, that the mycelium had been "externally applied" by the sheathing base of the uppermost leaf. It is often clear macroscopically that the diseased areas on the chaffs of the newly-emerged ear are precisely those upper and dorsal parts which were in contact with the diseased parts of the enwrapping sheath, *not* the embryonic ends which are tucked in towards the rachis. The group of grains which Ravn figured ((8), Taf. 1) as being typical of the early stages of infection show this fairly well. When the ears emerge the lesions on the grains may be broad and long-established or small and newly established, the reasons for this variation being exactly the same as the reasons for variation in the leaf stripes. This variation in the degree of chaff discoloration is important for the man who has to select barley to plant as seed.

#### 14. "BLINDNESS" IN LEAF-STRIPED BARLEY.

The ears of Leaf-stripe plants are commonly of the type known to the farmer as "blind" ears. In other words, they do not completely emerge from the sheathing base of the uppermost leaf. Nothing more than a summary of the reasons for this is required.

(1) The uppermost parts of the stem are weak. This weakness is contributed to both by the "externally applied" mycelium and by the mycelium which rots the core. In connection with the former it may be noted that the uppermost parts of the stem are commonly the most completely invaded, just as the uppermost leaves are. The uppermost internodes, because of this weakness, cannot play their part in thrusting the ear out from the confining sheath.

(2) The forces which retard the emergence of the ear from the sheath are also, in diseased plants, abnormally great, since the auricles of the uppermost leaf are hard and mummified, forming a barrier across the natural exit of the ear.

These two causes combine to produce the imperfect emergence of the ear. Ravn considered that such imperfect emergence is peculiar to Leaf-stripe disease (*H. gramineum*). A similar imprisonment of the ear may, however, result when *H. teres* causes "mummification" of the auricles of the uppermost leaf. Outside the genus, various fungi which affect the auricles in this way, and cause also a greater or less weakness of the straw, may produce this symptom.

## 5. THE DISEASE AND THE TILLERS.

The tillers which arise from a diseased main shoot may escape, may be killed, or may live infected to a varying extent. The re-iteration of these three possible fates may seem platitudinous but, in point of fact, one gathers from the existing accounts that the third of these fates is inevitably met. On general grounds alone the reader of the foregoing paragraphs will immediately question this. Our fungus is not one which inevitably allows to live any organ which it invades, nor is it one which inevitably succeeds in infecting an organ, even though the older organs which enwrap or ensheath that organ contain a well-established mycelium.

Speaking of the case of the tillers in more particular terms, one may note the following facts. The source of infection for the tillers which are produced on a diseased main shoot, is the mycelium contained in the sheath part of the leaf which subtends the tiller. (To a lesser extent, of course, any other diseased part with which the tiller may come into contact can contribute.) As to why mycelium from that source need not always be successful, various reasons might be advanced. The fungus may not have moved in the leaf to a position favourable for invasion of the new part. Or, again, the fungus, by reason of the competition of other micro-organisms, or the accumulation of its own waste products, may be in no condition to make new progress.

## 16. THE TILLERS WHICH ESCAPE.

The possibility of escape is most often realised in the case of late tillers. It is more important and hopeful, from the practical point of view, to note that tillers frequently escape early enough in the season for them to produce useful ears, so that in the field it is something of a shock to find, at the base of fine healthy shoots, the ruins of others killed by Leaf-stripe.

As to how often, in actual field observations, escape of tillers can be found, my experience differs from that of Ravn. He records<sup>(7)</sup> that of 396 diseased plants he found only 3 per cent. with any sound tillers. The percentages which I found were higher, *e.g.* in one series of observations, dealing with 215 diseased plants, I found that 42 per cent. had at least *some* tillers sound. Such discrepancies between his work and mine may partly be explained by the difference in the conditions of the experiments, *e.g.* as regards climate and type of seed.

## 17. THE TIME FOR OBSERVING THE INCIDENCE IN A CROP.

Probably, however, the main reason for the discrepancy is to be found in the fact that Ravn's count was made at one period of the season only, viz. soon after earing (7, p. 114). In such a count, the remains of tillers which have been killed by the disease, are very easily missed at the base of fine healthy tillers. Since this point concerning the time of counting is important from the point of view of getting correct results in infection experiments, for instance, or susceptibility trials, it may be added here that a count too early in the season is still more unreliable. Even for the purpose of classifying plants simply as infected or uninfected, it is not advisable to make a count before the barley plants have reached the "rosette stage." At earlier stages the disease, if not actually lagging out of sight, is present in atypical or inconspicuous forms. If the most accurate results are desired, a "rosette-stage" count should be supplemented by one taken about the time of earing.

## 18. INFECTION EXPERIMENTS.

From considerations of space, several questions concerned with artificial infection can be left over to a later paper, in which they can be discussed as being common to several species of the genus. Among these questions are the devising of efficient means of infecting the germinating grain, and also the whole topic of "secondary infections." Secondary infections of the leaf are not an important source of damage, so far as this disease is concerned, while secondary infections of the young grain have not been quite fully investigated.

*Disease-intensity under Varying External Conditions.*

Certain results, however, concerning the influence of conditions on the disease, results derived both from infection experiments and from general observations, are intimately connected with the main theme of this paper.

*Soil-temperature at germination.* Many observations of mine confirm what is already known from the results of Ravn (7, 8) and Johnson (3a), namely that when seed which harbours the fungus is subjected to low temperatures at the time of germination, the chances of a diseased plant being produced are greatly increased.

Low temperatures greatly slow down the germination, increasing the chance of the fungus effecting an entry while the young shoot is still confined within the chaffs. The development of all parts of the

plant may be similarly retarded by low temperatures during the later life of the plant. Thus temperature undoubtedly has a bearing on the question of how many plants will, completely or partially, escape. It may be noted that, according to Ravn, the fungus makes slow progress even at 3–5° C.

*A New Attitude Concerning the General Effect of Conditions.*

It has already been emphasised that a great difference between the course of the disease, as here described, and the Smut-like course which was ascribed to it by Ravn, lies in the fact that a plant, which has been infected in its early stages, must now be considered to be able, by vigorous growth, to grow away from the fungus. Sowing when the soil-temperature is relatively high (on British standards) is one way of promoting this vigour.

*Manurial treatment* immediately suggests itself as another means to the same end. Frew (2), dealing with a somewhat similar problem, found superphosphates to be the best accelerators for the growth of barley. Heavy applications of nitrogenous manures, it is well-known, produce a "lush" growth in barley, and observations show that crops which have received such applications are particularly liable to show severe *Helminthosporium* symptoms.

That conditions which influence the *number of tillers* produced by barley plants, conditions such as *manuring* and *density of sowing*, certainly have an effect on the incidence of the disease in a crop, follows from what has been said in earlier paragraphs concerning the escape of tillers, in particular from the statements concerning the superior chance of the later tillers escaping.

Factors such as these which I have just mentioned, which, unlike the soil-temperature factor, do not affect the proportion of the crop which initially becomes infected, but influence the proportion of the attacked individual which becomes diseased, may be termed minor factors. In actual practice these minor factors would probably be ignored, but it is not my opinion that farmers can afford to ignore the one thoroughly important result of this study of conditions, namely that *winter-sown barley in Britain is particularly liable to be attacked*. Special care in the disinfection of the seed is therefore essential. Even though this disinfection should fall short of the ideal, the farmer is entitled to expect, according to the new attitude very much more than according to the old, that good husbandry, producing vigorous plants, will counteract the effect of bad seed.

## 19. SUMMARY.

*The accepted view* has been that this fungus, in causing Leaf-stripe Disease, behaves as many Smut-fungi do, the mycelium spreading from the apical growing-point of the stem to the leaves and other parts.

It is *here claimed* that the leaves are first infected, and the stem-apex infected only in the final phase of the disease. Infection of the stem-apex is not a necessary preliminary to the production of a fully diseased plant. In fact, infection of that apex is, very often, followed swiftly by the death of the plant. This fungus cannot live as a "tolerated symbiont" in such growing-points.

For the proof of this main contention, as well as for the general increase of knowledge, stages in the life-history of affected plants are reviewed in turn.

The sources of infection for the germinating grain are described.

This is followed by evidence, revealed by the microscope, concerning the penetration from these sources, and the subsequent spread of the fungus.

The normal sequence of leaf symptoms, as also deviations from this sequence, provide useful evidence concerning the main thesis. By discussion of these (and of questions concerning the tillers) it is hoped that identification of cases of this disease is made easier.

Hitherto it has been considered that only while it remains outside the germinating seed is the fungus in an insecure position, at the mercy of circumstances such as temperature. The real importance of temperature at that time is here strongly reaffirmed, but, further, it is pointed out that the insecurity of the fungus in a Leaf-striped plant persists for a very much longer time. Vigorous shoots often grow away from the fungus. In this way, and in others, good farming has its reward in fighting this disease.

## REFERENCES.

- (1) DRECHSLER, C. (1923). Some Graminicolous Helminthosporiums. *Journ. Agr. Res.* xxiv, No. 8, 641.
- (2) FREW, J. G. H. (1924). On *Chlorops taeniopus*. *Ann. App. Biol.* xi, No. 2, 175.
- (3) JOHNSON, E. C. (1914). Some Imperfect Fungi collected from Oats, Wheat and Barley. *Journ. Agr. Res.* v, No. 1, 475.
- (3 a) JOHNSON, T. (1925). Studies on the Pathogenicity and Physiology of *H. gramineum*. *Phytopath.* xv, 12.
- (4) NOACK, F. (1905). *Helminthosporium gramineum* Rabenh. und *Pleospora trichostoma* Wint. *Zeits. Pflanzenkr.* xv, 193.

## 260 *The Helminthosporium diseases of Cereals in Britain*

- (5) VAN POETEREN (1922). Verslag over die werksamheden van den plantenziek. dienst, 1920 en 1921. *Versl. Meded. Plantenziek. Dienst Wageningen*, No. 27. (Review in *Rev. App. Myc.* 1923.)
- (6) RAUNKJAER, C. (1899). Die Danske Blomsterplanters. *Naturalhistorie*, 1, 534.
- (7) RAVN, F. KOLPIN (1900). "Nogle *Helminthosporium* arter og de af fremkaldte sygdomme hos byg og havre" (I Kommission hos Universitetsboghandler, Copenhagen.) Much the same in *Bot. Tidskr.* xxiii, 101, 1900.
- (8) — (1904). Ueber einige *Helminthosporium* und die von denselben hervorgerufenen Krankheiten bei Gerste und Hafer. *Zeits. Pflanzenkr.* xi, 1. [Though more easily accessible, this is by no means as full as (7).]
- (9) ROSTRUP, E. (1893). *Sygdomme hos Landbrugsplanter forarsagede af snyltesvampe*. Copenhagen. [And other reports quoted by Ravn (7).]
- (10) SMITH, N. J. G. (1924). The Parasitism of *Helminthosporium gramineum*. Abstract in *Proc. Camb. Phil. Soc. (Biol. Sci.)*, 1, pt. 2.
- (11) STEVENS, F. L. (1921). *Helminthosporium* and wheat foot-rot. Abstract in *Phytopath.* v, No. 11.
- (12) — (1922). The *Helminthosporium* foot-rot of wheat, with observations on the Morphology, etc. of *Helminthosporium*. *Illinois Nat. Hist. Surv. Bull.* vol. xiv.
- (13) VOGT, E. (1923). Ein Beitrag zur Kenntniss von *Helminthosporium gramineum* Rab. *Arb. Biol. Reichsanst. für Land- und Forstwirtschaft*, xi, No. 5, 387.
- (14) ZADE (1924). Neuere Untersuchungen über die Lebensweise und Bekämpfungen Haferflugbrandes. *Angewandte Botanik*, Bd. vi, Hf. 2.

(Received September 4th, 1928.)



# ON THE STEM ROT OR WILT DISEASE OF CARNATIONS<sup>1</sup>

By W. J. DOWSON, M.A., D.Sc.

(*Department of Agriculture, Launceston, Tasmania.*)

(With Plate XIII.).

## CONTENTS.

	PAGE
1. Introduction . . . . .	261
2. Brief review of previous work on carnation wilt diseases . . . . .	262
3. Occurrence and symptoms of the disease in carnation houses . . . . .	263
4. Greenhouse experiments and observations . . . . .	264
5. Examination of diseased plants . . . . .	265
6. The isolation of a number of <i>Fusaria</i> from diseased tissues . . . . .	265
7. Inoculation experiments . . . . .	266
8. Conclusions drawn from inoculation experiments . . . . .	269
9. The vitality of the conidia of <i>F. 6</i> and <i>F. 7</i> ( <i>F. culmorum</i> ) . . . . .	270
10. The production of toxic substances by <i>F. 6</i> . . . . .	271
11. The growth of the carnation <i>Fusaria</i> at various temperatures and their behaviour on different media . . . . .	272
12. The identity of the <i>Fusaria</i> isolated from diseased carnations . . . . .	274
13. The conditions under which <i>F. herbarum</i> and <i>F. culmorum</i> can bring about infection and the propagation of the disease . . . . .	275
14. Suggestions for the control of the disease . . . . .	276
15. Conclusions . . . . .	277
16. Summary . . . . .	277
References . . . . .	279
Explanation of Plate . . . . .	280

## 1. INTRODUCTION.

LOSSES among carnation growers due to a disease known as "stem rot" have only reached serious proportions since the War, although in other countries much trouble of a similar nature has been experienced for many years. At the instigation of the Director of the Royal Horticultural Society's Garden, Wisley, the author undertook an investigation with

<sup>1</sup> Part of thesis submitted for the degree of D.Sc., University of London.

## 262 *On the Stem Rot or Wilt Disease of Carnations*

the object of finding out (1) whether or not the disease occurring in English nurseries is the same as that recorded elsewhere, (2) under what conditions the disease occurs and is spread, and (3) what control measures could be recommended to carnation growers.

### 2. BRIEF REVIEW OF PREVIOUS WORK ON CARNATION WILT DISEASES.

The tree or perpetual flowering carnation was originally raised in France about the year 1840 but was later introduced into America and has since become known as the American carnation. It is from the latter country that the great majority of stocks grown in England have been derived.

In 1897 Sturgis<sup>(1)</sup> described a carnation disease generally known as "die-back" or "stem rot" the symptoms of which are very similar to those noted in the present investigation. Sturgis isolated a *Fusarium* which he did not name, but which was capable of bringing about similar symptoms when introduced into sterilised soil. He also showed that the disease could be spread by infected cuttings.

In 1898 Stewart<sup>(2)</sup> showed that Sturgis was probably dealing with two distinct diseases of which the "stem rot" was due to *Rhizoctonia* and involved a sudden wilting, and the "die-back" to a *Fusarium* which acted relatively slowly.

In 1900 Delacroix<sup>(3)</sup> published the result of a full investigation of a similar wilting disease which had caused considerable losses to growers in the south of France. He attributed the disease to a *Fusarium* which forms chlamydospores under certain conditions and which he named *Fusarium dianthi* P. and D. In a paper closely following this, Mangin<sup>(4)</sup> gave reasons for not accepting this name and stated that the *Fusarium* concerned was that hitherto known as *Fusarium roseum* Link.

In 1915 van der Bijl<sup>(5)</sup> investigated what is known as "wilt" or "crown rot" of carnations grown in the open in South Africa (Natal). This was of the sudden wilt type described by Stewart<sup>(2)</sup>, involving a soft rot of the cortex at the base of the stem the xylem of which was stained brown. He isolated a *Fusarium* which on inoculation produced the disease and which in pure culture gave rise to a yellowish colour rather than a pink or rosy one and formed chlamydospores readily. Van der Bijl also showed that high temperatures and much moisture were favourable to the incidence and spread of the disease.

In 1920 Small<sup>(6)</sup> investigated a wilt of carnations and other plants in Uganda exactly similar to that described by van der Bijl in South Africa. A *Fusarium* was isolated the growth characters of which were in close

agreement with those given by van der Bijl, and which in a subsequent paper Small<sup>(7)</sup> gives reasons for identifying as *Fusarium udum* Butler, the cause of pigeon pea wilt in India.

### 3. OCCURRENCE AND SYMPTOMS OF THE DISEASE IN CARNATION HOUSES.

The disease is most in evidence during the months of June, July and August when the general average temperature is higher for a longer period than at any other time of the year. Diseased two and three years old plants are then most conspicuous; but it is not uncommon for 18 months old plants and even cuttings, freshly transplanted, to show signs of wilting due to disease.

A characteristic symptom is the slow withering with loss of colour of the shoots one after another. The green colour gradually changes to a pale straw yellow and serves to distinguish such plants from others which wilt more suddenly from some other cause such as wireworm damage. If allowed to remain in the beds the wilt progresses until every part above ground is affected. The roots decay and under moist conditions the cortex of the collar becomes soft and rotten and bear pustules of *Fusarium* spores. The name of stem rot commonly employed by growers is due to this symptom, which, however, in the author's opinion is by no means constant. The disease is primarily a wilt.

During this investigation another trouble was frequently encountered both in growers' houses and in the experimental house at Wisley. This was a form of die-back which is included here because it was found that the same fungi are involved in both stem rot and die-back. From time to time a number of plants were observed with one or more young shoots dying back from the topmost node. Investigation showed that these shoots had been "stopped" by having their upper portions "pinched out," and that a few weeks later they had commenced to die back. This process goes on slowly for many weeks and even months; but so far I have not found that the rest of the plant becomes affected, the dead portion extending no further than the junction with the larger branch or main stem.

On more than one occasion freshly struck cuttings from different sources have been observed with similar wilting symptoms (Plate XIII). A considerable number of such plants die if the conditions of moisture and temperature remain unaltered. When both are diminished a certain number recover and become healthy plants. Investigation showed that two species of *Fusarium* were involved, one of which was distinct from those concerned in stem rot and die-back.

## 4. GREENHOUSE EXPERIMENTS AND OBSERVATIONS.

In the course of the investigation a rather striking fact was once observed in a nursery situated on heavy clay, and as it was intimately connected with local conditions and the prevalence of disease in certain beds and even particular portions of a bed it is recorded here in some detail.

In one of the older types of houses a patch of wilted plants occurred in the middle of every bed. The patches together formed a straight line at right angles to the length of the house; upon enquiry it was ascertained that an underground stream ran in the same direction. The disease was always present in these spots the soil of which throughout the year was much wetter than elsewhere, thus indicating that amongst the conditions favouring the disease a relatively high moisture content was important.

In the experimental house an attempt was made to imitate this state of affairs by dividing an improvised bed into two parts by a concrete partition. One half of the bed was made up with very shallow soil, not more than 4 in. deep, while in the other half the soil was nearly 2 ft. deep. Both were planted at the same time with different varieties of carnations, one half bed being the duplicate of the other in this respect. The half bed with deep soil was over-watered, while the other half was kept as dry as was consistent with good growth. During the 18 months which followed two things became apparent: (1) that the plants on the shallow dry soil were not much less in stature than those in the deep wet soil; and (2) that a much greater number of plants succumbed in the latter than in the former. The beds were made up with ordinary garden soil to which no infectious material had been added.

The observations were considerably interfered with by the attacks of mice which gnawed through a number of plants in the wet bed and by an attack of red spider in the dry bed. In spite of these complications considerably more plants presented the characteristic wilting and yellowing symptoms of the disease in the wet bed than in the dry one. An examination of the wilted plants showed mycelium in the lower parts (see below) and from some of them species of *Fusarium* were subsequently isolated.

This experiment would seem to confirm the observation made above, viz. that the stem rot disease is more prevalent under relatively moist soil conditions.

Among many growers the opinion is prevalent that deep planting favours the disease and that plants with the upper portions of their roots exposed are not so liable to attack.

## 5. EXAMINATION OF DISEASED PLANTS.

Attention was concentrated upon the stem rot or wilt disease as being more serious than either die-back or wilting of the cuttings. Microscopical examination was made of plants of different ages and in different stages of attack. Moreover, by the courtesy of Dr H. H. Storey a consignment of plants received from Natal was also examined.

In the English material mycelium was found in the stems just above ground level and in the upper parts of the roots of plants having only a few wilted shoots. The most satisfactory stain for revealing the presence of mycelium was found to be "Cotton Blue" dissolved in lactophenol, applied in the manner described by Klebahn<sup>(8)</sup>. The collar of the plants contained more mycelium than any other part and nearly all its tissues from the epidermis to the pith were invaded by intra- and extra-cellular hyphae. Above and below this region the hyphae were more abundant in the xylem than in the other tissues, while at the very edge of the infected parts only a few hyphae could be seen in the vessels. It was a little surprising to find mycelium nearly an inch from the crown in the xylem of roots which appeared outwardly quite healthy.

Another striking fact observed in connection with the mycelium was the large amount of a gum-like substance in both tracheids and vessels, generally in the neighbourhood of the hyphae. In fact, in the roots and collar the presence of this substance, conspicuous in longitudinal sections, almost invariably indicated the position of the hyphae, some of which were actually within the gum-like mass. The majority of these embedded hyphae consisted of dead and empty cells, but a few retained the deep blue of the stain and had the appearance of being alive.

In tracing exactly how far the mycelium had reached and to what extent the actual wilting of the shoots was due to its presence, the bases of wilted shoots were examined just beyond their insertion with the main stem, but in no instance was mycelium found in such parts. On the other hand, much of the xylem was blocked up with the gum-like substance mentioned above.

## 6. THE ISOLATION OF A NUMBER OF FUSARIA FROM DISEASED TISSUES.

By planting pieces of tissue taken from diseased zones containing mycelium on to agar plates *Fusarium* was almost invariably obtained. A large number of isolations were made from fresh material derived from various sources and were kept in pure culture on various agar media such as Dox's, potato broth and artichoke (see below).

From some wilted cuttings a strain of *Fusarium* designated *F. 1* was isolated. From young wilted plants sent to the author a *Fusarium*, *F. 2*, slightly differing from the above in growth characters was obtained. This strain after a few months in pure culture on Dox's agar suddenly produced two sectors in a Petri dish with rosy pigmentation, one of which was isolated as *F. 3*. As however, subsequent inoculation experiments indicated that neither *F. 2* nor *F. 3* was parasitic they were discarded. Another strain, *F. 4*, was isolated from the dead portion of a stem of a still living plant. A fifth strain, *F. 5*, was obtained from two young diseased plants in the experimental house at Wisley. One of these showed typical stem rot lesions at the collar and had practically no roots when lifted. Pieces of the collar when kept over night on damp filter paper developed salmon coloured spore masses from which isolations were made. In the other plant the disease was of the die-back type and the fungus isolated from the wilted shoot proved to be *F. 5*.

Finally in July 1927, from plants 18 months old, obtained from a nursery in Kent, showing unmistakable signs of the wilt form of disease, *F. 6* and *F. 7* were isolated. These subsequently proved to be identical.

The material received from Natal was in too mouldy a condition for an adequate examination to be made; but in one plant the grey-brown streak in the main stem observed by van der Bijl<sup>(5)</sup> and Small<sup>(6 and 7)</sup> was found. No *Fusarium* however, was isolated from this plant but only a species of *Verticillium* which proved to be non-pathogenic.

## 7. INOCULATION EXPERIMENTS.

Inoculation experiments were carried out with the six strains mentioned above. Plants 12 or 18 months old were generally used for this purpose, but some recently struck cuttings were also inoculated. The inocula consisted of either a suspension of conidia in sterilised distilled water or mycelium plus a little agar. In the earlier inoculations the inocula were introduced into wounded internodes or leaf axils, or the "pinched out" internodes of young and vigorous shoots. In later experiments wounded older branches and the main stem at the collar were inoculated. The wounds were afterwards covered with cotton wool or tinfoil. One series of inoculated plants was kept in a cool greenhouse, another in a corner of the orchid house and a few in a glass incubator made to the author's design and kept in a large bay window of the laboratory.

The following are selections of the inoculations made:

*A. Inoculations with F. 1 (F. avenaceum, see below).* A young healthy plant having five upright shoots the tops of which were "pinched out" by hand was inoculated with spores and mycelium as follows: On 5. xi. 26 all the exposed nodes received a drop of distilled water. To two of them one loopful each of a rich spore suspension was added, two more received a small piece of mycelium on agar, and the fifth served as a control. The five shoots were then covered with tinfoil. The plant was placed in a

cool greenhouse. On 16. xii. 26 a very slight discoloration was observed below the tin-foil covering one of the inoculated shoots, the rest showed no change, and no further development took place.

Using the same inocula two similar plants were inoculated through wounds at the base of the main stem; the plants remained quite healthy.

A similar plant was treated as the plant first inoculated, but was kept in the laboratory in the sink and covered with a bell jar. In a few days a rich white growth developed over the wounded shoots which at the end of a month had died back to the next node. The atmosphere under the bell jar was fairly warm ( $25^{\circ}$  C.) and very moist.

It was concluded from this and similar experiments that *F. 1* at ordinary temperatures was not parasitic, although at relatively high temperatures and in a moist atmosphere, such as sometimes obtain in propagation houses, this *Fusarium* is probably capable of causing some damage.

*B. Inoculations with F. 2, F. 3 and F. 4.* Under ordinary conditions inoculations with these strains had no effect so they were discarded.

*C. Inoculations with F. 5 (F. herbarum, see below).* On 26. i. 27 pieces of mycelium of *F. 5* were introduced into four slightly wounded internodes. A fifth internode wounded in the same way was not inoculated. All were covered with tinfoil, and the plant placed in the cool greenhouse. After eight days the first definite sign of infection was observed on one of the four inoculated shoots. A slight change of colour to a paler green than normal was quickly followed by very slight withering and shrivelling, the tips of most of the leaves exhibiting a few longitudinal depressions or striations due to contraction. On 9. ii. 27 another inoculated shoot exhibited similar symptoms, and on 15. ii. 27 a third shoot showed signs of infection. This shoot had been inoculated much further down into more woody tissue. The shrivelling of the leaves above the inoculated wound commenced at the base and spread towards the tips. On 19. ii. 27 the fourth shoot inoculated low down like the preceding became infected. On 17. iii. 27 all the inoculated shoots were dead, shrivelled and straw coloured above the wounds. The infection did not spread downwards past these places and the control shoot remained healthy.

On 21. i. 27 using a rich suspension of spores from a culture of *F. 5* a plant was inoculated in four places, viz. on the tops of two "pinched out" shoots and into two wounded leaf axils. All were covered with tinfoil and the plant kept in the glass incubator at a temperature of about  $24^{\circ}$  C. On 3. ii. 27 one of the inoculated shoots wilted and withered, and after 16 days all four shoots showed signs of infection and subsequently withered. In none did the infection travel down the stem.

On 7. iii. 27 six shoots of a well grown plant were inoculated through incisions and afterwards covered with tinfoil. Two of the shoots were inoculated with mycelium and one with spores, both grown at  $24^{\circ}$  C. The other three received spores or mycelium grown at a temperature of  $27^{\circ}$ – $30^{\circ}$  C. No infection took place with either spores or mycelium produced at the higher temperature, but the other three shoots became infected in from five to nine days. From these and similar experiments it was concluded that *F. 5*, which had been originally isolated from the die-back form of disease, was an active causal agent of the disease which was involved under conditions of moderate temperature ( $24^{\circ}$  C.) and moisture.

*D. Inoculations with F. 6 and F. 7 (F. culmorum, see below).* On 12. vii. 27 a large plant was inoculated in the following manner: the upper portions of three stems

## 268 *On the Stem Rot or Wilt Disease of Carnations*

were cut off and the stumps moistened with drops of distilled water. On one cut surface a little mycelium of *F. 6* was placed, on another the spores from a culture of *F. 7* were added and the third stem served as a control. The plant was stood in a dish containing a little water and the whole was covered with a bell jar in the laboratory at a temperature of about 26° C. A pink and white mycelium rapidly developed over the inoculated shoots but not over the control. By the end of August about three inches of each inoculated shoot had died back. On removing the bell jar and placing the plant in the cool greenhouse the die-back ceased.

A small seedling plant about six inches high was inoculated at soil level through a slit in the stem with mycelium from *F. 6*. The slit was pressed together and kept closed by damp soil. The plant was placed in a sink in the laboratory under a bell jar, which was removed after one month. On 8. viii. 27 the lowest leaves had turned yellow and there were slight signs of wilting above. On 15. viii. 27 the lowest leaves had become brown and shrivelled and the wilt above was now quite definite. On 30. viii. 27 the condition of the plant resembled that seen in growers' houses.

A similar seedling plant treated as above but placed in the orchid house and not under a bell jar was not infected.

A similar plant treated in the same way but in the cool greenhouse was not infected.

The above series seems to indicate the importance of moist conditions at the place of inoculation for infection to take place.

On 30. vii. 27 two three-year old plants, both with several upright branches, were inoculated with conidia from *F. 7* by introducing a loopful of a suspension of spores into wounded leaf axils. Five leaf axils of each plant were slightly wounded with a sharp sterilised scalpel, and, of these, four on each plant were inoculated and one on each was left as a control. After inoculation each wound was tied round with one twist of thick string. Both plants were placed in the orchid house. Definite signs of infection were noted on 8. viii. 27 which became more pronounced on 11. viii. 27; by 30. viii. 27 two to three nodes below each inoculation had been killed. The controls remained healthy. This experiment shows that *F. 7* (*F. culmorum*) is able to produce the die-back type of disease.

On 11. viii. 27 a three-year old plant was inoculated with conidia from *F. 7* introduced as a rich suspension into a deep incision at the base of the main stem. The incision was closed by one twist of thick string and the plant placed in the orchid house. After 19 days much of the foliage was wilting and pink spore pustules were observed at the edge of the wound. The plant became completely withered in about a month. *F. 7* was re-isolated from this plant.

On the same date a similar plant treated in the same way but kept in a cool greenhouse did not become infected.

On the same date a one-year old plant treated in the same way was placed under a bell jar in the laboratory. Three months elapsed before there were any signs of wilting, but by December the plant was dead.

On the same date a similar plant treated in the same way but kept in the orchid house was dead after four months with the stem rotted through at ground level.

This series not only indicates the necessity for moisture at the place of inoculation but that older plants are more rapidly affected than young ones. Furthermore, *F. 7* is capable of producing the stem rot type of disease.



On 17. viii. 27 the soil around two young seedlings in separate pots, raised from sterilised seed in sterilised soil, was moistened by pouring 10 c.c. of a suspension of *F. 7* conidia down the collar of each plant. One was kept in the cool greenhouse and the other in the orchid house. Both were killed by December. This again indicates that young plants take longer to succumb to the disease than older plants.

On 14. ix. 27 a three-year old plant was inoculated at the base of the stem about  $1\frac{1}{2}$  in. from the soil through a deep slit with conidia of *F. 6*. An inverted waxed paper cone was fixed round the stem at soil level and contained water in order to keep the inoculated place moist. The plant was kept in the laboratory and well watered. On 5. xi. 27 there were slight indications of infection and the plant was moved into the greenhouse on 17. xi. 27. The paper cone was kept filled with water the level of which did not quite reach the inoculated wound. By January 1928 the whole of the aerial portion had wilted and withered. In this instance infection first became evident after an interval of about seven weeks. A similar plant without the paper cone kept in the greenhouse the whole time was not infected. The infected plant was then examined. The portion above the wound was dead and pink spore pustules were present around the edges of the cut. The plant, however, remained firm in the ground and the roots were not infected at the time the examination was made. Abundant mycelium was found in all tissues except the pith between soil level and the wound. The cortex of this region was soft and rotten and resembled a case of typical stem rot.

On 1. xi. 27 a couple of two-year old plants were inoculated with conidia through incisions at the base of the stems about one inch above soil level. Each wound was wrapped round with thick string one end of which dipped into a vessel containing water. In this way the wounds were kept continually damp without the exclusion of air. Both plants were kept on the laboratory bench in a good light. After three weeks a shoot of one plant commenced to wilt. The youngest leaves did not open out but remained clasped within the older ones, which became paler in colour. Striations soon appeared upon the leaves which finally shrivelled. After six weeks from the start all the shoots of both plants had wilted and withered but both remained firm in the soil. On 21. i. 28 both stems parted from their roots about  $\frac{1}{2}$  in. below soil level. A control plant remained unaffected.

On 17. xi. 27 a two-year old plant was inoculated at the base of the stem with mycelium from *F. 6*. The wound was covered with damp muslin and wrapped round with string the end of which dipped in water. On 21. xii. 27 one shoot wilted and dried up; shortly afterwards growth entirely ceased and the plant slowly withered. On 19. i. 28 the plant was lifted and examined. The roots were sound. A red discoloured area was present in the wood about the wound and a narrow strip of brown wood extended as far as the roots. Mycelium was present from the wound in the stem to the base of the first wilted shoot but was not abundant. Much gumming was found everywhere except at the collar (below the wound) and tyloses were numerous.

## 8. CONCLUSIONS DRAWN FROM INOCULATION EXPERIMENTS.

From these inoculation experiments the following conclusions were drawn:

(1) The carnation disease investigated by the author is not due to *Fusarium udum* which causes a similar disease in Africa (7) characterised

## 270 *On the Stem Rot or Wilt Disease of Carnations*

by sudden wilting, early death (within three weeks), and a dark discoloration of the wood.

(2) There are at least two *Fusaria* intimately concerned.

(3) One of these, *F. 5*, is a causal agent involved in the die-back type of trouble under conditions of high temperature and moisture, while the other, *F. 6*, under similar conditions will bring about both die-back and stem rot.

(4) A third *Fusarium*, *F. 1*, under the same conditions may be weakly parasitic to shoots.

(5) Plants 18 months to two years old are affected more rapidly with the wilting type of disease than are either younger or older plants.

(6) The *Fusaria* involved gain entrance through wounds.

(7) Both spores and mycelium of *F. 5* produced at 27° to 30° C. did not bring about infection, but similar material grown at 24° to 25° C. was able to do so.

### 9. THE VITALITY OF THE CONIDIA OF *F. 6* AND *F. 7* (*F. CULMORUM*).

The following observation is of some interest as concerning the vitality of spores under adverse conditions. The spore suspension of *F. 6* and *F. 7* used in some of the inoculations and kept in watch glasses covered with a Petri dish were examined periodically for a period of three months. The great majority of spores sank to the bottom in a very short time but a few remained on the surface. Nearly all the latter germinated within 12 hours, but the former remained dormant and only germinated when laid on the surface of agar or tap water, or when they reached the surface owing to the evaporation of the water in the watch glasses. The loss from this cause was made good from time to time by the addition of a little tap water. After three weeks to one month it was found that the sunken spores had changed in appearance. The two terminal cells (the spores were mostly 6-celled) seemed to be empty and dead while the four inner cells had become swollen oval bodies with thick walls, still colourless and with granular contents. In some instances only one or two of the inner cells had behaved in this manner while all the remaining cells appeared dead and empty.

This is the only instance of the formation of resting cells or chlamydo-spores which the author has observed in this particular *Fusarium* which under ordinary conditions of culture did not produce such bodies. When placed on agar the thick oval cells germinated in the course of 12 hours or so and continued to do so up to the end of three months when the contents of the watch glasses became contaminated with other organisms.

The importance of this observation lies in the possibility of introducing the disease through a contaminated water supply as Bewley<sup>(9)</sup> has pointed out.

#### 10. THE PRODUCTION OF TOXIC SUBSTANCES BY *F. 6*.

As wilting associated with more or less definite yellowing of the foliage has been shown in other diseases to be due to the presence of toxic substances in the transpiration current, Aster wilt<sup>(10)</sup>, sleepy disease of tomatoes<sup>(11)</sup>, and the silvering of foliage due to *Stereum purpureum*<sup>1</sup>, attempts were made to ascertain if the same was true of the carnation disease. The following experiment was set up. Small flasks were filled with about 20 c.c. of the following liquids:

(a) The liquid from a culture of *F. 6* grown in Dox's solution, filtered through a Chamberland candle.

(b) A similar portion of sterilised Dox's solution.

(c) The liquid from steamed carnation stems in sterilised water.

(d) The liquid from a culture of *F. 6* grown on steamed carnation stems in sterilised water, filtered through a Chamberland candle.

(e) Tap water.

(f) Sterilised distilled water.

Into each flask two vigorous shoots about 8 in. high were placed. After four days the leaves of the shoots in (a) were slightly paler in colour and had wilted somewhat, and striations due to contraction were present at the bases of most of the leaves. No change in colour was observed in the shoots of (b) but the leaves had wilted to about the same extent and striations were present. A few striations were present on the leaves of the shoots in (c). The shoots in (e) and (f) were normal. After a fortnight the shoots in (a) and (d), both in Fusarium liquid, showed considerable differences as compared with the others chiefly in the *yellowing* and withering of the lower leaves and in more extensive shrivelling.

The first sign of the effect of the Fusarium-liquid was a slight change of colour, the affected leaves and stems changing from normal green to pale green and finally pale yellow. This was followed by shrivelling which in the leaves generally commenced at the base and progressed onwards to the tips. While the leaves were still only pale green slight longitudinal depressions were formed and increased in number until the leaves appeared to be striated.

<sup>1</sup> The author wishes to thank Mr F. T. Brooks for a verbal communication of this interesting fact.

Inoculated and infected shoots cut off in the pale green stage and placed in water partially recovered, but similar shoots in the pale yellow or straw coloured stage did not recover when so treated. Transverse sections of such stems fixed in alcohol showed that the chlorophyll tissue of the cortex had more or less completely collapsed into a series of ridges and depressions resting upon the collenchymatous cylinder of the stem. In some instances the chlorophyll tissue had been reduced to a brown strip of squashed cells bringing the unaffected epidermis almost against the collenchymatous cylinder. The chlorophyll had become disintegrated into an amorphous yellow mass.

This experiment appears to indicate that toxic substances *are* produced as the result of the presence of the mycelium within the xylem and are taken up in the transpiration current to the chlorophyll tissue which is thereby killed.

In another experiment cut shoots were placed in two test tubes, one containing water, the other being empty. The shoots in the latter *wilted* in a few hours, the tips bending over the rim of the tube. There was no visible loss of colour for 12 days by which time the stem and the leaves had shrivelled with the formation of longitudinal striations similar to those seen in infected shoots. Finally the shoots became completely withered and straw coloured and were then indistinguishable from withered infected shoots. The shoots in water remained practically unchanged. The experiment is an instance of a true *wilt* due entirely to the withholding of water and which is distinguished from the wilt of *infected* shoots by the absence of loss of colour as a *first* symptom.

#### 11. THE GROWTH OF THE CARNATION FUSARIA AT VARIOUS TEMPERATURES AND THEIR BEHAVIOUR ON DIFFERENT MEDIA.

The Fusaria were grown mostly on Dox's and potato broth agar. Other media such as steamed carnation stems in sterilised water, artichoke agar, Brown's asparagin agar and sterilised plugs of potato or carrot were also employed. On all except that of asparagin agar both *F. 5* and *F. 6* produced abundant aerial mycelium, mostly white, but sometimes streaked with pink or yellow, or both, with a characteristic rosy tint in the substratum, most marked on artichoke agar, and least so on Dox. On artichoke agar the colour was so intense that the glass itself was slightly tinted and remained so after washing in hot water. *F. 1* did not produce any colour in the substratum of any of the media, but much white aerial mycelium. Salmon to orange coloured spore pustules were

formed after about a month on most media with the exception of asparagus agar on which the growth of all was relatively feeble.

A series of incubators ranging from 15° C. to 40° C. and varying by 5° C. was placed at the disposal of the author by the Imperial College of Science, by means of which the optimum and maximum temperatures for growth of these *Fusaria* were determined. A double series of plates containing Dox's agar inoculated with the *Fusaria* was run for about a fortnight and repeated two or three times. The increase in the average diameter of the growth measured daily was taken as indicating the daily rate of growth at each temperature (Brown(12)). The analysis of the figures so obtained showed that the optimum temperature for growth of the three *Fusaria* is the same, *i.e.* about 25° C. and that the maximum for all three lies between 35° C. and 40° C.

A further series of incubators run with differences of 2° C. between 15° C. and 30° C. and between 34° C. and 40° C. indicated that the best temperature for growth was about 26° C. and the maximum about 38° C. for the three *Fusaria*. Mr F. T. Brooks kindly made the necessary arrangements for growing the same *Fusaria* at the Cambridge Low Temperature Research Station in which inoculated slants of Dox's agar were placed at - 6° C., - 1° C. and 0° C. to + 1° C. These tubes were examined after an interval of six months when it was found that no growth had taken place at - 6° C. *F.* 1 (*F. avenaceum*) had produced much aerial growth and spore pustules at - 1° C., while the other two had not grown at this temperature. All had produced very appreciable growth and spore pustules at 0° C. to + 1° C. *F.* 5 and *F.* 6 formed a rosy tint in the substratum at this temperature.

The three *Fusaria* were also grown on Dox's agar slants rendered acid, neutral and alkaline by the addition of either sulphuric acid or caustic soda and brought to a definite *pH* value by means of the colorimetric method. On the acid side the *pH* value was 3.6 and on the alkaline 8.8. By the addition of two drops of universal indicator to the tubes while still hot, shaking and then allowing them to set, the changes of colour (and therefore of *pH*) could be observed during the growth of the fungi when compared with control tubes. The acid medium was pale pink and on it the three *Fusaria* grew very slowly at first. The colour of the medium gradually changed to pale blue, but not so blue as was the alkaline medium. Both *F.* 5 and *F.* 6 grew best on the neutral medium which also became slightly alkaline as indicated by the appearance of a very pale blue tint. On the alkaline medium the growth was a little less vigorous and the colour changed slightly towards the neutral. *F.* 1 grew best on

the alkaline medium the colour of which turned to rather a deeper blue than that indicated by the control tubes, and on the acid and neutral media the same tint was finally produced.

From these observations it was concluded that *F. 1* prefers a more alkaline medium than do either *F. 5* or *F. 6* which grow best on the alkaline side of neutrality. At the time these cultures were made the identity of the *Fusaria* was not known and the writer was much interested to receive Dr Wollenweber's determinations, for the observations are in close agreement with those of Lundegärth<sup>(13)</sup> who happened to work with the same three species (*F. avenaceum*, *F. herbarum* and *F. culmorum*).

## 12. THE IDENTITY OF THE *FUSARIA* ISOLATED FROM DISEASED CARNATIONS.

The author is fully conscious of the difficulties in naming *Fusaria*, particularly in view of the work of Brown<sup>(14)</sup> and Hansford<sup>(15)</sup>, and of his own experience of the appearance of saltants in cultures of *F. 2* (see above). Cultures were sent to Dr Wollenweber at Berlin who was kind enough to name the three *Fusaria* as follows: *F. 1* = *Fusarium avenaceum* (Fries) Sacc., *F. 5* = *Fusarium herbarum* (Corda) Fries, and *F. 6* and *F. 7* = *Fusarium culmorum* (W.G.S.) Sacc.

In letters to the author Dr Wollenweber pointed out how very widespread these three species were in Nature and that they occurred on a large number of plants belonging to different families; further, that *F. avenaceum* and *F. herbarum* could be regarded as varieties of the same fungus, so hard was it to distinguish them on morphological grounds.

For biological reasons however, as the present work shows, they can be fairly easily separated, for *F. avenaceum* is a saprophyte at ordinary temperatures, does not produce pigment on Dox's agar and grows well at  $-1^{\circ}\text{C}$ ., whereas *F. herbarum* is a wound parasite on carnation shoots, produces a rosy colour in Dox's medium and will not grow at  $-1^{\circ}\text{C}$ .

A considerable number of more or less well defined species of *Fusarium* have been recorded as occurring upon carnations, a list of which is given by Wollenweber in the new edition of Sorauer's *Handbuch der Pflanzenkrankheiten*. Lewis<sup>(16)</sup>, also quoted by Wollenweber, was able to produce a rot of carnation buds with a number of species none of which had been isolated from carnations but which had been obtained from apples, grasses, etc.

According to Wollenweber, the species which Delacroix<sup>(3)</sup> worked with and called *F. dianthi* P. and D. may have been *F. aurantiacum*

(I.k.) Sacc. (*Elegans* group), which is also very widespread on many different plants.

It seems clear from previous investigations(7 and 16) and from the present work that there are a number of more or less well defined species of *Fusarium*, widely spread in Nature, which under certain conditions can invade and kill the stem tissues of carnations.

### 13. THE CONDITIONS UNDER WHICH *F. HERBARUM* AND *F. CULMORUM* CAN BRING ABOUT INFECTION AND THE PROPAGATION OF THE DISEASE.

The present investigation indicates that the presence of a relatively high moisture content of both soil and atmosphere, and a relatively high temperature (24°–26° C.) are important factors in relation to infection, and that these fungi can only gain entrance through wounds.

Lundegärth(13) has shown that a high (3 to 7 per cent.) concentration of carbon dioxide in the soil favours the growth of these *Fusaria* and leads to the attack of wheat seedlings. The very general occurrence of *F. avenaceum* in most soils is due, according to Lundegärth, to its tendency to produce a slightly alkaline reaction (see above), and the development of plant diseases in which *Fusaria* are concerned is favoured by plentiful applications of organic manures whereby the carbon dioxide content of the soil is increased, thus favouring the growth of these fungi.

The die-back type of the disease (in which "pinched out" shoots are involved) can probably be found in any nursery where the watering is rather overdone during the summer months, as both *F. herbarum* and *F. culmorum* are probably present in the soil of the beds at the very start, and both form spores on dead and decaying material. Infection is almost certainly due to the splashing of the spores on to the "pinched out" internodes during watering.

The exact place of infection in the stem rot form has not been definitely ascertained, but it is certainly through wounds due to various causes such as wireworm attack, and the natural cracking of the cortex of the collar of some varieties (Plate I). It is possible that *F. culmorum* may commence its attack as a die-back of one or more "pinched out" shoots and then pass down to the older parts of the plant, ending up at the roots. The experiments recorded here, however, do not support this view as in no instance of experimentally produced die-back did the stem rot form result. It is of course possible that the somewhat greater care with which the relatively few plants at Wisley were cultivated as compared with the many thousands in any nursery may have had something

to do with the possible arrest of the die-back form of disease. In support of this, attention may be called to the incidence of disease in the over-watered bed in the Wisley experiments and the absence of naturally occurring disease in potted plants in the same house. Here, the two factors of ventilation and moisture are involved as the pots were spaced at much wider intervals than were the plants in the beds which were the usual 8-in. apart either way. There are some varieties of carnations very prone to the stem rot disease, which when grown in contaminated soil are very liable to become infected. But besides the planting of susceptible varieties in soil already containing *F. culmorum* there is the additional risk of actually carrying the parasite along with its host when young plants are sent from one nursery to another. To save freight charges and space, plants as small as possible are transported and are generally recently potted up cuttings which have only been knocked out of their pots ("sixties") and are sent with the soil still surrounding the roots. Should this soil contain traces of *F. culmorum* it is almost certain to infect that of the new nursery, though infection of the plants probably does not take place, as has been shown, until the plants are much older.

Bewley (9) has shown that some water supplies contain *Fusarium* and other spores which are capable of introducing disease into nurseries, and the author has demonstrated that *F. culmorum* can form dormant cells when kept in water, which will germinate after three months.

#### 14. SUGGESTIONS FOR THE CONTROL OF THE DISEASE.

As the three *Fusaria* concerned are widespread and probably occur in most soils, the best means of control is to sterilise the beds, preferably by steam, before planting. This, however, would be useless if cuttings were planted to the roots of which contaminated soil was adhering. The difficulty could be overcome in two ways: either by raising one's own plants, "struck" in sterilised sand; or by having a quarantine house in which all plants received from outside sources could be grown in sterilised soil for a period of at least two years.

Apart from soil sterilisation, every care should be taken in watering to avoid splashing the "stopped" plants and to keep the beds on the dry side rather than the reverse. Furthermore, the temperature should be kept as low as possible by ventilating and shading during the summer months. The possibility of introducing the disease through contaminated water should not be overlooked.

Among many growers the opinion is prevalent that deep planting is closely connected with the disease and that plants having the bases of



their roots slightly exposed are less often attacked. This may be connected with the natural cracking of the cortical tissues of the collar of certain varieties, but the point calls for further investigation.

### 15. CONCLUSIONS.

The chief conclusion to be drawn from the present investigation is that the English disease is not due to the same causal agents as in other countries, with the possible exception of the American die-back disease defined by Stewart(2).

The occasional wilt of very young plants in England is due to *F. culmorum* (W.G.S.) Sacc. The fairly common die-back of "stopped shoots" is due to *F. herbarum* (Corda) Fries and *F. culmorum* (W.G.S.) Sacc.; while the far more serious stem rot or wilt is due to *F. culmorum* (W.G.S.) Sacc., the only species so far met with in this type of disease.

The author would like to express his thanks to Prof. V. H. Blackman for advice and criticism throughout the investigation and to Dr Brown for his kindness in arranging for cultures to be grown at various temperatures at the Imperial College. To Mr F. T. Brooks his thanks are also due for kindly arranging to have cultures grown at the Cambridge Low Temperature Research Station. The author's thanks are also due to Dr Wollenweber, of Berlin, for naming the three *Fusaria* with which this paper deals.

### 16. SUMMARY.

1. Previous investigations of carnation diseases, attributed to *Fusaria*, are briefly described and the scope of the present work is outlined.

2. The disease is most conspicuous in carnation houses during the height of summer and is characterised by a progressive wilting and withering of the shoots which eventually assume the colour of straw. Sometimes the cortex at the base of the main stem becomes rotten, but the disease is primarily a wilt. A die-back of "stopped" shoots was found to be fairly common.

3. Observations indicated the importance of much soil moisture as a factor in the incidence and prevalence of the disease.

4. Mycelium in the tissues of diseased plants was found mostly at the base of the stems, in all tissues, but was confined to the xylem above and below this area. In plants in the last stages of disease mycelium also occurred at the base of the shoots. The xylem of the upper portions of roots, outwardly healthy, was also found to be infected. Some hyphae

## 278 *On the Stem Rot or Wilt Disease of Carnations*

were seen to be embedded in a gum-like substance of which there was a great deal. The wood of recently wilted shoots contained much gum but no hyphae.

5. Isolations from diseased material gave *Fusarium*. The wilted cuttings yielded a strain of *Fusarium*, *F. 1*. *F. 2* was isolated from young wilted plants and in culture produced a pink sector or saltant, *F. 3*. *F. 4* was obtained from a similar source and *F. 5* from two plants, one of which showed the stem rot type of disease, and the other the die-back form. *F. 6* was obtained from the basal portion of an 18 months old plant exhibiting the wilting or stem rot type. *F. 7*, which proved to be identical with *F. 6*, came from the same source. Specimens from Natal only yielded a *Verticillium* which proved to be non-pathogenic.

6. Spores or mycelium were introduced into wounds in the nodes, internodes and collars of seedlings, 18 months and two years old plants under different conditions of temperature and humidity.

7. Under conditions of relatively high temperature and humid atmosphere, *F. 1* can act as a weak parasite to shoots. *F. 2*, *F. 3*, and *F. 4* proved to be non-pathogenic. *F. 5* caused die-back only and *F. 6* and *F. 7* caused both die-back and stem rot. Plants 18 months to two years old are more rapidly affected than are either younger (seedlings) or older ones. The *Fusaria* can only bring about infection through wounds.

8. The spores of *F. 6* germinate at once in drops of water or on agar, but sink to the bottom in water of any depth, do not germinate unless brought to the surface, and form a few thick-walled colourless resting bodies. These germinate within 12 hours when sown on agar, and such altered spores retain the power of germination for at least three months.

9. Experiments with filtered liquid in which *F. 6* had been growing and in which carnation shoots were placed showed that a toxic substance was conveyed by the transpiration current to the chlorophyll tissues which were killed. No gumming was produced in such shoots and the first sign of wilting is a slight loss of colour produced in from four to five days. True wilting due to the withholding of water takes place in a few hours without loss of colour.

10. On solid media *F. 1* produced abundant white aerial mycelium, while *F. 5* and *F. 6* formed white aerial hyphae streaked with pink and sometimes yellow, with always a rosy pink colour in the substratum.

The optimum temperature for growth was about 26° C. and the maximum about 38° C.

11. *F. 1*, *F. 5*, and *F. 6* were identified by Wollenweber as *F. avenaceum* (Fries) Sacc., *F. herbarum* (Corda) Fries, and *F. culmorum* (W.G.S.) Sacc., respectively.

12. The conditions under which infection takes place are a relatively high temperature (24°–26° C.), high humidity and the presence of wounds. Deep planting also probably favours infection.

13. Sterilisation of the beds is recommended as the best means of control.

14. In England the occasional wilting of quite young plants is due to *F. culmorum* while the much commoner die-back of "stopped shoots" is caused by both *F. culmorum* and *F. herbarum*, and the more serious stem rot or wilt is due to *F. culmorum*.

## REFERENCES.

- (1) STURGIS, W. C. (1897). Preliminary Investigation on a Disease of Carnations. *Ann. Rep. Conn. Agric. Exp. Sta.* III, 175–181.
- (2) STEWART, F. C. (1898–99). Stem Rot Disease of Carnations. *Exp. Sta. Records*, vol. x.
- (3) DELACROIX, G. (1900). La Maladie des Oeillets d'Antibes. *Ann. Nat. Agron.* XVI, 161–201.
- (4) MANGIN, L. (1900). Sur la Maladie des Oeillets d'Antibes. *Comptes rendus hebdomadaires des Séances de la Société de Biologie*, LII, 248.
- (5) VAN DER BIJL, P. A. (1916). "Wilt" or "Crown-Rot" Disease of Carnations caused by *Fusarium* sp. *Ann. App. Biol.* II, 267–291.
- (6) SMALL, W. (1920). A Wilt of Carnations, *Nigella*, *Delphinium* and *Cosmos*, with a Note on *Sclerotium Rolfsii*. *Kew Bulletin*, pp. 321–328.
- (7) — (1923). On the Occurrence of a Species of *Fusarium* in Uganda. *Kew Bulletin*, pp. 269–291.
- (8) KLEBAHN, H. (1910). Krankheiten des Selleries. *Zeitschr. f. Pflanzenkrankh.* XX, 23.
- (9) BEWLEY, W. F. (1921). On the Fungus Flora of Glasshouse Water Supplies in Relation to Plant Disease. *Ann. App. Biol.* VIII, 10–19.
- (10) DOWSON, W. J. (1922). On the Symptoms of Wilting of Michaelmas Daisies produced by a Toxin secreted by a *Cephalosporium*. *Trans. Brit. Myc. Soc.* VII, 283–286.
- (11) BEWLEY, W. F. (1922). "Sleepy Disease" of the Tomato. *Ann. App. Biol.* IX, 116–134.
- (12) BROWN, W. (1925). Studies in the Genus *Fusarium*. II. An Analysis of Factors which determine the Growth-Forms of certain Strains. *Ann. Bot.* XXXIX, 373–408.
- (13) LUNDEGÄRTH, H. (1923). Die Bedeutung des Kohlensäuregehalts und der Wasserstoffionkonzentration des Bodens für die Entstehung der Fusarien. *Bot. Notiser*. I. Abstract in *Rev. App. Myc.* vol. II, 1923, p. 382.
- (14) BROWN, W. (1926). Studies in the Genus *Fusarium*. IV. On the Occurrence of Saltations. *Ann. Bot.* XL, 223–243.
- (1928). Studies in the Genus *Fusarium*. VI. General Description of Strains, together with a Discussion of the Principles at present adopted in the Classification of *Fusarium*. *Ann. Bot.* XLII, 285–304.

280 *On the Stem Rot or Wilt Disease of Carnations*

- (15) HANSFORD, C. G. (1926). The Fusaria of Jamaica. *Kew Bulletin*, pp. 257-288.  
(16) LEWIS, C. (1913). Studies of Disease-producing Species of Fusarium. *Maine Agr. Exp. Sta. Bull.* 219.

EXPLANATION OF PLATE XIII.

Wilting carnation cuttings with cracks at the collar.

(Received October 19th, 1928.)





## THE INFLUENCE OF BRIGHT SUNSHINE UPON THE TOMATO UNDER GLASS

By W. F. BEWLEY, D.Sc.

*(Experimental and Research Station, Cheshunt.)*

(With 5 Text-figures.)

GLASSHOUSE crops, in common with those grown in the open, vary from year to year in accordance with the weather. Some measure of protection is afforded by the screen of glass and artificial heat, but the necessary mechanical control of light, heat and humidity has not developed sufficiently to counteract external conditions. Some figures have been obtained at Cheshunt during the past eleven years, which suggest an intimate connection between the weight of the tomato crop and the amount of bright sunshine occurring during the season. They are considered of sufficient interest to warrant publication, although it is realised that many points require the confirmation which only prolonged investigation can provide.

### THE RELATION BETWEEN CROP YIELD AND SUNLIGHT.

A definite fluctuation in annual yield was first noticed in 1920, when graphs were prepared from the records of the tomato experiments. The fluctuation was so striking that confirmation was sought on commercial nurseries in the district.

The graphs comprising Fig. 1 were prepared by plotting the years along an abscissa, and the crop weight in tons per acre along an ordinate. They illustrate the fluctuation in crop yield from 1917 to 1927 inclusive as occurring in four different nurseries *A-D* and in tomato house 2 at the Experimental Station. Details are given in Table II. Nurseries *A*, *B* and *D* are situated in the Lea Valley, and Nursery *C* near East Grinstead. It has been difficult to obtain records from nurseries because only blocks of houses which have received the same treatment over a period of years and in which the same variety had been cultivated during that time could be used. With one exception, the nurseries employed fulfilled these requirements, records being taken from blocks of houses about half an acre in extent. Nursery *B* was included because certain soil treatments produced differences which are readily observed.

Perhaps the most striking feature of the graphs in Fig. 1 is their approximate uniformity. Some differences are shown by Nursery B, where treatment of the soil with cresylic acid in 1920, and steam in 1922 and 1926, increased the yield. In 1923 an attack of *Verticillium wilt* conveyed in contaminated manure depressed the yield abnormally. The yield from the experimental house 2 was also increased in 1927 by steam sterilisation.

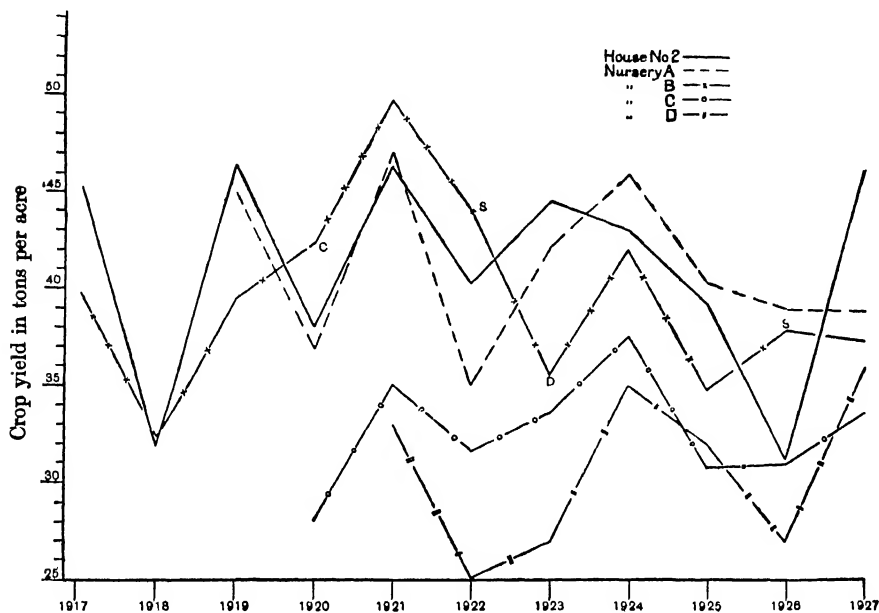


Fig. 1.

The peak year, 1921, suggested a reason for the variation in yield, because this year is remembered by all as one of exceptional sunshine.

Weather records for Cheshunt were obtained from the Meteorological Office, and those for the Surrey nursery from Mr W. G. Franks of the Observatory, Brockhurst, East Grinstead, to whom our grateful thanks are due. No figures for Cheshunt were available: they were calculated from those obtained at Rothamsted, Benington, Enfield and Tottenham, being the four nearest stations around Cheshunt. Details are given in Table I.

Fig. 2 depicts the curve of total hours of bright sunshine from April 1st to August 31st inclusive, this period being chosen because experience indicates that sunshine is especially valuable during these months. It is



less important during January to March when high temperatures stimulate leaf and stem development at the expense of the roots.

It will be seen that the sunshine curve follows the same trend as that

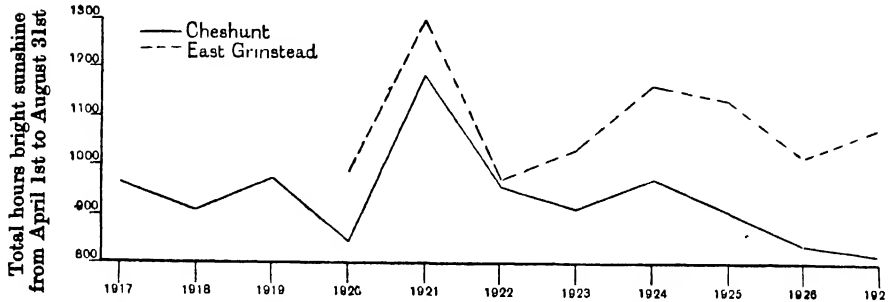


Fig. 2.

relating to crop yield with peaks in 1917, 1919, 1921 and 1924, and depressions in 1918, 1920, 1922, 1923, 1925, 1926 and 1927, the last being the exception at East Grinstead. The data from East Grinstead shows a

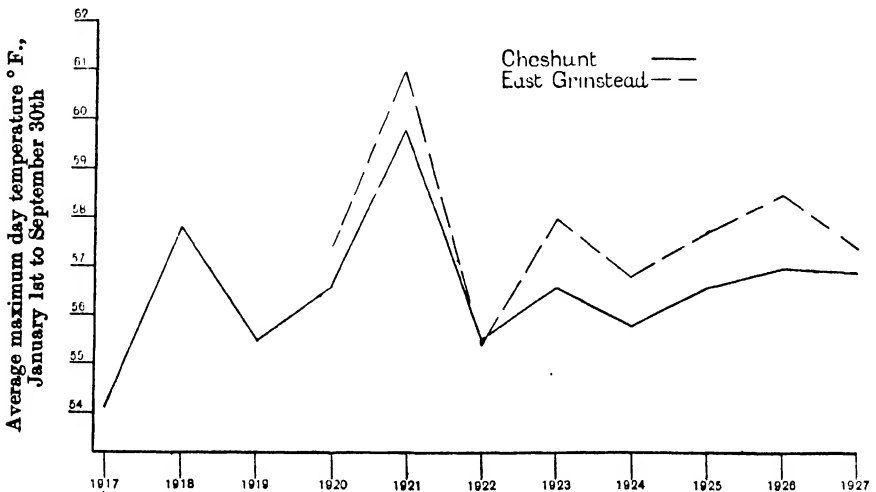


Fig. 3.

marked relationship between bright sunshine and crop yield. Similar results are described by Tippet<sup>1</sup> from observation of the wheat yields at Rothamsted.

<sup>1</sup> Tippet, L. H. C. "On the Effect of Sunshine on Wheat Yield at Rothamsted." *Journ. of Agr. Sci.* xvi, pt. 2, 1926.

Rainfall and temperature might be expected to affect the plant through its entire life. The graph of average maximum day temperature from January 1st to September 30th inclusive, and that for total rainfall during the same period are given in Figs. 3 and 4 respectively. It will be seen that, while these factors undoubtedly affect the total weight of crop produced, they are not so important as bright sunshine.

#### THE RELATION OF MANURIAL TREATMENT TO SUNSHINE.

The weather also exerts a strong influence on the type of growth—and for successful cultivation of a rapidly growing plant like the tomato under all conditions, the cultivator must know how to alter not only his temperature and humidity, but also his manurial treatment in accordance with prevailing weather conditions.

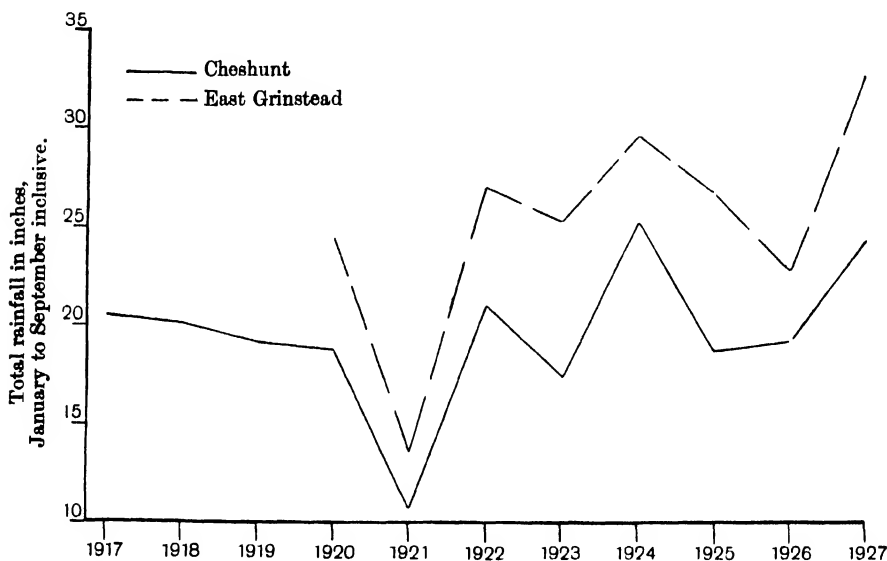


Fig. 4.

An attempt has been made to obtain some information on this point by studying the records of manurial plots in combination with meteorological conditions. In Fig. 5, the crop weight from four different plots during a period of eleven years is shown graphically, ordinary data being shown in Table III. The plots are situated in house 3, Series J, at the Cheshunt Station, and the treatment of each plot has remained constant since 1916. One plot has received complete artificials, and in three others, nitrogen, phosphates and potash respectively have been omitted

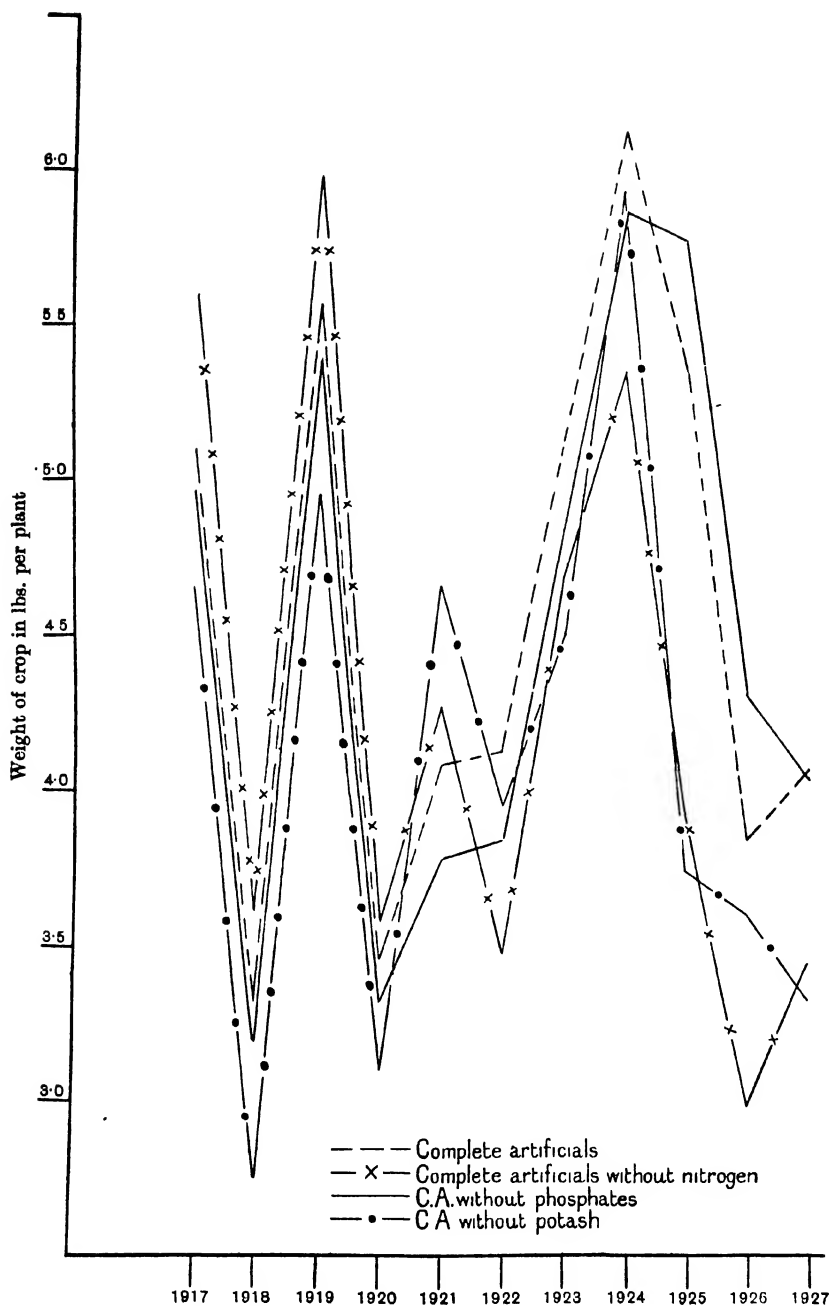


Fig. 5.

## 286 *Bright Sunshine upon the Tomato under Glass*

each year. The house was steamed in 1924, and succeeding years must be omitted from the present discussion.

Considering the years 1917 to 1923, the relation between crop yield and sunshine can again be noted. An interesting point is the uniformity of the graphs illustrating the different plots, up to 1921, when the complete artificials without potash plot, which had previously given the lowest yield, suddenly rose to be the highest. This at once suggests that prolonged sunshine has a similar effect on the plant to that produced by potash manures, the omission of which had previously caused a relatively low yield.

The practical conclusion from these experiments, first suggested in 1921<sup>1</sup>, is that the tomato crop requires less potash in fine sunny summers than during dull weather when it is most valuable, and that the amount of nitrogenous fertilisers must be increased during sunny weather. Observation on commercial nurseries has confirmed this and improved crops have been obtained by manuring in accordance with these conclusions.

### SUMMARY.

1. The tomato crop produced in glasshouses is affected by prevailing weather conditions.
2. The yield per acre varies directly in relation to the total hours of bright sunshine during the period April 1st to September 30th.
3. Total sunshine also affects the potash requirement of the tomato, less potash being required during bright sunny weather than under dull conditions.

Table I.

### *Meteorological Data.*

Year	Total hours bright sunshine, April to August inclusive		Average maximum day temperature, ° F., January to September inclusive		Total rainfall in inches, January to September inclusive	
	Cheshunt	E. Grinstead	Cheshunt	E. Grinstead	Cheshunt	E. Grinstead
1917	963.9	—	54.1	—	20.5	—
1918	909.8	—	57.8	—	20.1	—
1919	971.9	—	55.5	—	19.1	—
1920	843.4	985.9	56.6	57.4	18.8	24.5
1921	1184.1	1289.0	59.8	61.0	10.7	13.6
1922	957.1	971.1	55.5	55.4	21.1	27.1
1923	911.0	1042.1	56.7	58.0	17.5	25.3
1924	972.3	1164.2	55.8	56.8	25.4	29.7
1925	908.8	1134.4	56.6	57.7	18.9	26.8
1926	838.2	1019.4	57.0	58.5	19.4	22.9
1927	788.1	1076.3	56.9	57.1	24.6	33.2

<sup>1</sup> *Seventh Annual Report of the Experimental and Research Station, Cheshunt*, p. 14, 1921.

Table II.

*Tomato crop in tons per acre.*

Year	Nursery A	Nursery B	Nursery C	Nursery D	House 2
1917	51.2	39.7	—	—	45.3
1918	52.5	32.3	—	—	31.9
1919	56.0	39.5	—	—	46.4
1920	54.1	41.3	28.0	—	37.0
1921	53.8	49.7	35.0	33.0	46.4
1922	46.0	44.0	31.6	25.1	40.4
1923	46.5	35.5	33.6	27.0	40.6
1924	46.3	42.0	37.6	35.0	41.3
1925	41.2	34.8	30.8	32.0	39.2
1926	40.2	37.8	31.2	28.0	31.2
1927	48.5	37.3	33.6	36.0	46.4

Table III.

*Tomato crop in lbs. per plant from manurial plots.*

Year	Complete artificial	Complete artificial (less nitrogen)	Complete artificial (less potash)	Complete artificial (less phosphate)
1916	4.45	5.40	5.40	4.50
1917	5.11	5.60	4.65	4.98
1918	3.32	3.62	2.76	3.20
1919	5.57	5.98	4.95	5.38
1920	3.45	3.57	3.11	3.33
1921	4.08	4.27	4.65	3.78
1922	4.13	3.47	3.96	3.85
1923	5.09	4.69	4.51	5.01
1924	6.12	5.34	5.92	5.87
1925	5.34	3.92	3.75	5.77
1926	3.84	2.98	3.61	4.31
1927	4.07	3.44	3.33	4.06

*(Received October 30th, 1928.)*

## THE BIOLOGY OF THYSANOPTERA WITH REFERENCE TO THE COTTON PLANT

### IV. THE RELATION BETWEEN THE DEGREE OF INFESTATION AND SURFACE CAKING OF THE SOIL

By ELSIE I. MACGILL, M.Sc.

(*University of Manchester.*)

(With 2 Text-figures.)

DURING the summer of 1926 experiments were carried out concerning the influence of the water supply of a plant upon the degree of infestation of that plant by *Thrips*(2). As a result of these experiments a suggestion was made that "one factor concerned is the influence of heavy water supply upon the texture of certain soils in promoting surface caking which will act inimically to soil pupating species of Thysanoptera"; and in 1927 it was decided to devote special attention to this point.

The variety of cotton used in the following experiments was the Webber strain of American Upland Cotton used in the second series of experiments in 1926(2). Four blocks of cotton plants, *A*, *B*, and *X*, *Y*, were taken, each consisting of about 30 plants grown in pots of 23 cm. diameter and arranged as in the diagram (Fig. 1).

The shortest distance between any of the blocks of cotton was approximately 120 cm. and it has been shown in a previous paper(2) that at even shorter distances than this, there is very little migration of thrips from one block to another; any insects which did migrate were almost certainly adult thrips which had already oviposited; but for this reason, and also because the adult insect is much more active than the larva and is therefore more likely to be lost during the counts, the number of larvae per unit area of foliage gives a much more exact indication of the degree of infestation.

In all cases the soil—a medium clay loam which easily became caked—and the value of water supply—800 c.c. per pot per week—were the same, but in the case of blocks *A* and *B* the soil was never allowed to cake, the surface to a depth of 2–3 in. being kept in a state of fine tilth, while in blocks *X* and *Y* the soil was left undisturbed. The texture of the

surface soil in the pot did not seem to affect the health of the cotton plants, as blocks *Y* and *B* contained, on the whole, larger plants than the other two blocks. As in the previous season the plants were singularly free from infestation by insects other than thrips; apart from one or two plants slightly attacked by an aphid species, a few isolated individuals of

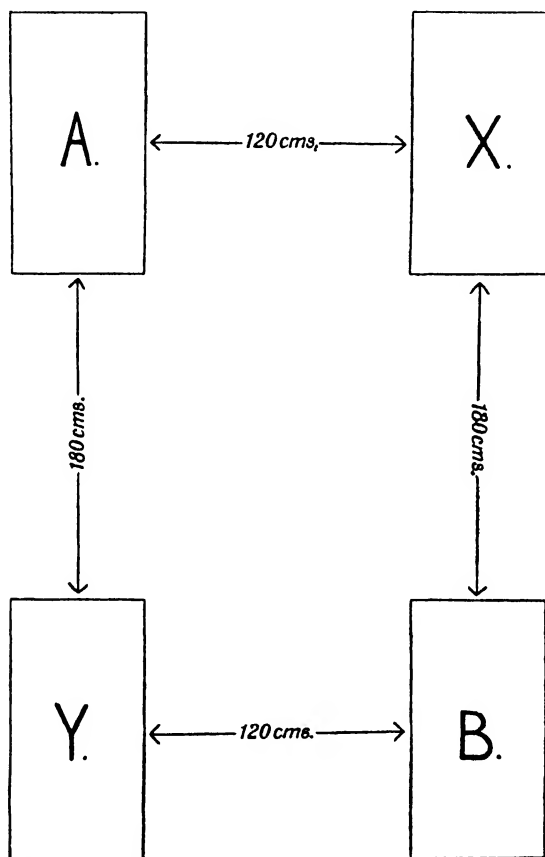


Fig. 1. Diagram showing the arrangement of the four blocks of cotton plants.

white-fly and leaf-hoppers were the only other insects noticed on the plants. Spiders and a species of predaceous mite occurred on all the blocks of plants but did not appear to be sufficiently numerous to decrease the number of thrips to any material extent.

Infestation counts of the thrips were made at intervals of a few days in the manner described in an earlier paper<sup>(2)</sup> and the infestation factor for each block thus obtained.

If the figures for the 1927 experiments are compared with those for 1926, two differences become apparent:

(1) The infestation factors for 1927 are considerably higher than the factors for 1926.

The highest factor for the whole period obtained in 1926 was 36.6 thrips per 100 sq. cm. of leaf surface, while in 1927 the highest factor was 65.3 thrips per 100 sq. cm., and three of the four blocks had an infestation factor greater than 36 thrips per 100 sq. cm. of leaf surface.

(2) On the whole the size of the leaves was less in 1927 than in 1926.

The second of these two differences can be explained by the fact that in the second series of experiments the plants were receiving a slightly smaller supply of water, so there was no tendency for the plants to form large leaves, as they do when the water supply is larger than necessary. The higher infestation factor in 1927 is explicable when a comparison is made of the temperature and humidity of the glasshouse for the two periods. In 1926 the mean temperature for the time during which counts of thrips were made was 19° C. (max. 26.7° C., min. 11° C.) and the mean relative humidity for the same period was 83.5 per cent. In 1927 the mean temperature for the corresponding period was 21° C. (max. 29.5° C., min. 11.9° C.) and the mean relative humidity 72.6 per cent., so that in 1927 the glasshouse conditions were a little more favourable for the multiplication of *Thrips tabaci* than in 1926(1).

The mean infestation for each of the four blocks of cotton plants for the period during which counts were made was as follows:

A. 65.3 thrips per 100 sq. cm. of leaf surface.

B. 42.8 thrips per 100 sq. cm. of leaf surface.

X. 39.4 thrips per 100 sq. cm. of leaf surface.

Y. 21.6 thrips per 100 sq. cm. of leaf surface.

Except for a short time at the beginning of the counts, block A, which was one of the blocks in which the surface soil was tilled, showed decidedly the greatest infestation by thrips, while block Y, in which the surface soil was allowed to become caked, was the least infested of the four blocks. The other two blocks, B (tilled soil) and X (caked soil), did not show such a marked difference, but as B showed a slightly higher infestation than X, the result is in agreement with the former one. If A and B are taken together as one block, and X and Y together as a second, the difference in the degree of infestation by thrips between blocks of plants with the surface soil tilled and those in which the soil was left undisturbed, is very marked (Fig. 2).



Mean infestation factor of *A* and *B*—54 thrips per 100 sq. cm. of leaf surface.

Mean infestation factor of *X* and *Y*—30 thrips per 100 sq. cm. of leaf surface.

The difference between the infestation factors for the two blocks was almost entirely due to differences in the numbers of larvae on the plants, the respective numbers of adult insects obtained from each block only differing slightly; the mean adult infestation factor of *A* and *B* was

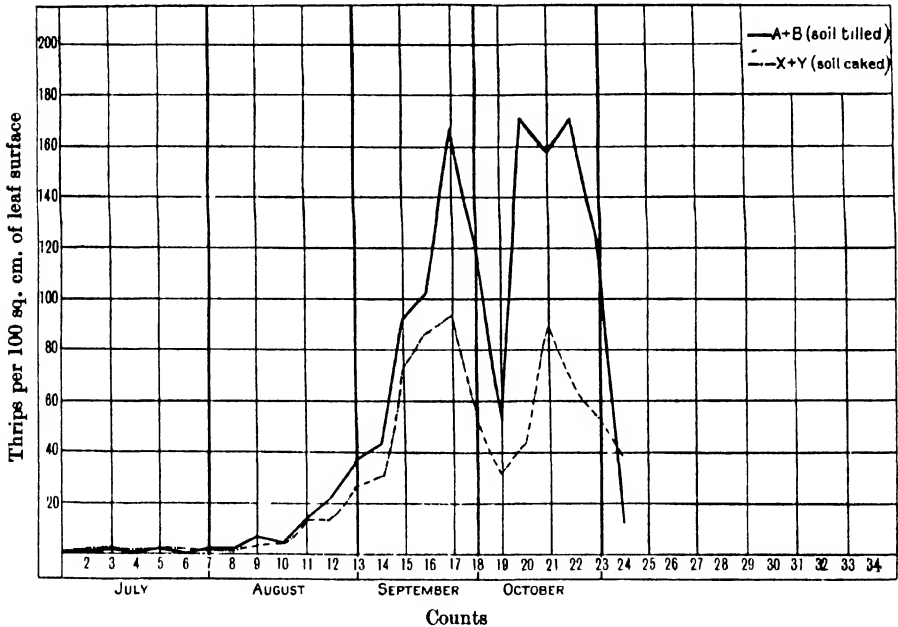


Fig. 2. The degree of infestation of two blocks of cotton plants; *A* + *B* with tilled, and *X* + *Y* with caked surface soil.

7 thrips per 100 sq. cm. of leaf surface, and the mean adult infestation factor of *X* and *Y* was 6.5 thrips per 100 sq. cm. of leaf surface, but it has already been pointed out that the adult infestation factor is very much less reliable than the larval one, as there is more likelihood of the adult insects being lost during the counts.

The highest infestation factor for adults plus larvae obtained during the counts was 255 thrips per 100 sq. cm. of leaf surface, which occurred on block *A* about the middle of October; the highest larval infestation factor (243 thrips per 100 sq. cm.) occurred on the same block at the same

time, but the highest adult factor, though occurring on the same date, was from block *X*, where 43.6 adults per 100 sq. cm. of leaf surface were counted.

The mean number of thrips per leaf for each block was:

<i>A.</i> 38 thrips per leaf.	<i>B.</i> 32 thrips per leaf.
<i>X.</i> 30 thrips per leaf.	<i>Y.</i> 24 thrips per leaf.

The largest actual number of thrips per leaf was counted at the end of September on block *Y*, when 347 thrips were obtained from one leaf, the largest number of larvae (328) was from the same block during the same count, though block *A* at the same time had one leaf with almost as many larvae (316). The largest number of adult insects (66) on a single leaf occurred on block *X* in the middle of September. The highest total number of thrips at any one count (10 leaves) was 1565 thrips from block *A* at the end of September—the largest number of larvae (1468) being on the same block at the same time; block *X* again had the largest number of adult insects per count, this was 267 thrips and was obtained in the middle of September.

The actual maximum number of thrips thus occurred at the end of September and after this there was a very decided fall in the number of insects. In October the glasshouse, which had not been heated artificially since the middle of July, was heated and shortly after this the number of thrips began to increase. When the figures for temperature and humidity were examined, it was found that after the glasshouse was heated the mean weekly temperature was slightly higher and the mean weekly relative humidity about 10 degrees less, so that the increase in the number of thrips seemed to be directly due to the higher temperature and decreased humidity, but on examining the graphs for the 1926 experiments, the same bimodal curves are found without any corresponding variations in the temperature and humidity. It seems probable, therefore, that the two peaks of the curve, especially as the time between them is approximately three weeks<sup>(1)</sup>, simply represent succeeding generations of the thrips and that the fall in the number of insects is caused by the majority of them being at that time in the prepupal and pupal stages, and therefore not found on the plants. The earlier generations do not show so plainly on the graph, and this may be partly due to a lower post-reproduction mortality of adult thrips under the more favourable conditions in the early part of the season, and also because of the much smaller number of insects involved.

The results of these experiments agree with the conclusion arrived at

during 1926, that one effect of a heavy water supply is to promote surface caking of the soil, and that this is unfavourable to soil pupating species of thrips.

I should like to take this opportunity of thanking Prof. J. S. Dunkerly for his helpful criticism and advice, and also Miss A. O. Martin, M.Sc., for her assistance in counting the thrips.

#### SUMMARY.

1. Results of experiments on the effect of water supply on the infestation of a plant by thrips suggested that the surface caking of the soil has an important influence on the degree of infestation.

2. Plants grown in pots in which the surface of the soil was tilled showed a higher infestation by thrips than plants in similar pots, but in which the soil was allowed to cake, although both sets of plants were receiving an equal water supply, and all other conditions were the same.

3. The difference in the infestation factors for the two groups was largely due to differences in the numbers of larval thrips, but it is pointed out that the number of larvae forms a more reliable factor than the number of adult insects.

4. The present experiments support the suggestion put forward that surface caking of the soil acts inimically to soil pupating species of thrips.

#### REFERENCES.

- (1) MACGILL, E. I. (1927). The Biology of Thysanoptera with reference to the Cotton Plant. II. The relation between temperature and life-cycle. *Ann. App. Biol.* XIV.
- (2) WARDLE, R. A. (1927). The Biology of Thysanoptera with reference to the Cotton Plant. I. The relation between the degree of infestation and the water supplied. *Ann. App. Biol.* XIV.

(Received July 26th, 1928.)

## A RECORDING SCALE FOR BEE HIVES

BY D. M. T. MORLAND, M.A.

*(Rothamsted Experimental Station, Harpenden, Herts.)*

(With 4 Text-figures.)

THE use of a hive on scales, the weight of which is recorded at least daily during the season, is strongly to be advised in any apiary. Unfortunately such a procedure is seldom adopted in this country. Continental bee-keepers, on the other hand, have their "Ruche sur bascule" or their "Wagstock," and many look upon it as part of the necessary equipment of the apiary. It enables them to keep an eye on the progress of the colonies and of seasonal effects thereon, and also serves as a guide in planning their work among the bees. Fortunately neither great sensitiveness nor extreme accuracy is essential for this purpose. The main object is to note the tendency of the weight changes, whether it be towards an increase or a decrease.

The most convenient type for general use is a platform scale large enough to take a single-walled hive of the dimensions used in the apiary, so that the centre of gravity of the hive may be over the centre of the platform. Both hive and scale should be protected from rain and from wind. Apart from the question of deterioration of the balance, due to exposure to the weather, it is surprising to note the weight of water added to a hive by a shower of rain. In windy weather it is difficult to weigh hives in the open because they sway about. The weight should be taken before or after the day's work, preferably before flying commences for the day. Readings taken at odd times of day may be very misleading. At the apiaries of the Dominion Experimental Farms of Canada, daily readings are taken at 7 a.m.

For the scientific investigator, greater sensitiveness is necessary, and some sort of recording mechanism is a great help. Mr C. B. Williams, formerly Government Entomologist at Cairo, has devised such an instrument(4). Recording scales have been used by Parks in Texas(3), and by the Bureau of Entomology at Washington. Williams used a platform scale having a pen on the end of the steelyard, which writes on a revolving drum such as is used in meteorological instruments. Employing

a really high-class scale, this arrangement should prove extremely useful for research work.

A recording balance, now in use at the Experimental Apiary at Rothamsted, although not so neat as the instrument to which reference has just been made, is giving satisfactory records, and has the advantage of being extremely sensitive. A simple scale beam *B* carries the hive and weights. Close behind this is a board, and mounted on a bracket is the clock drum *D* on which is the chart for the day. A pen *P* of the type generally used in meteorological instruments is fixed to an upright on

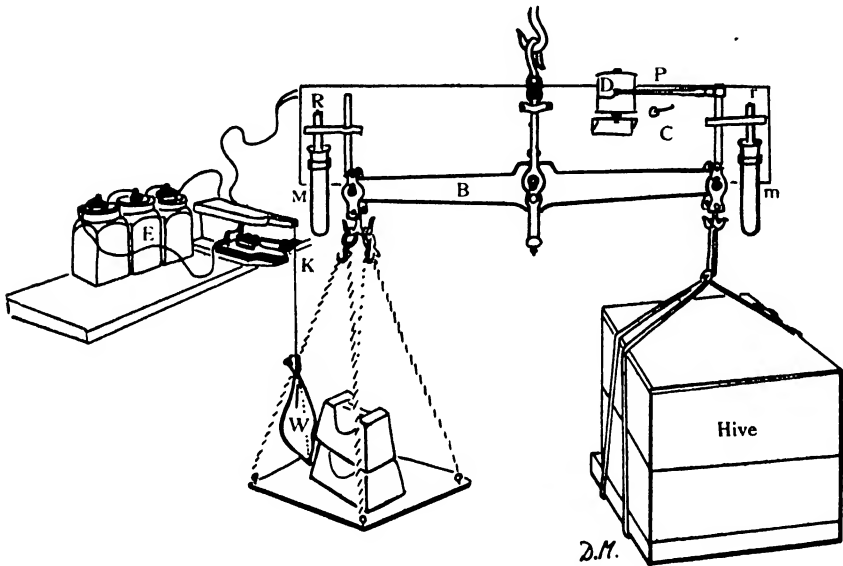


Fig. 1.

one of the stirrups of the balance, and records the changes of weight upon the drum. In order to steady the balance, two glass rods *R*, *r* are clamped in a vertical position at the ends of the beam. These dip in cups of mercury *M*, *m* supported on the board at the back. An upward pressure is exerted on the end of the beam, equal to the weight of mercury displaced by the rod. When the beam is inclined, the pressure is increased at the lower end, and correspondingly relaxed at the higher. By using larger rods in summer and smaller ones in winter, the range included on the chart can be adjusted to suit the expected variations in weight. (This principle is borrowed from Mr Williams' apparatus<sup>1</sup>.)

<sup>1</sup> It has been suggested that two vertical helicoid springs in tension would serve the same purpose.

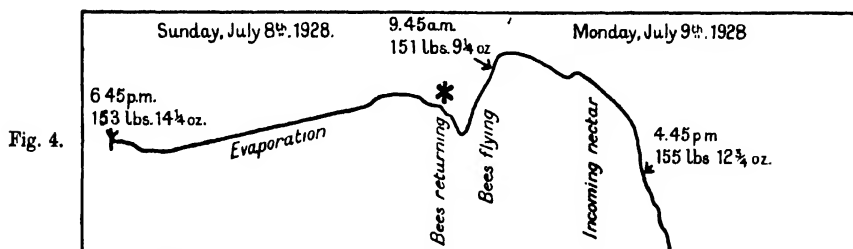
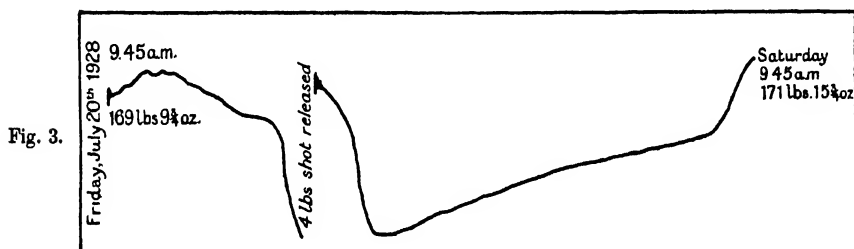
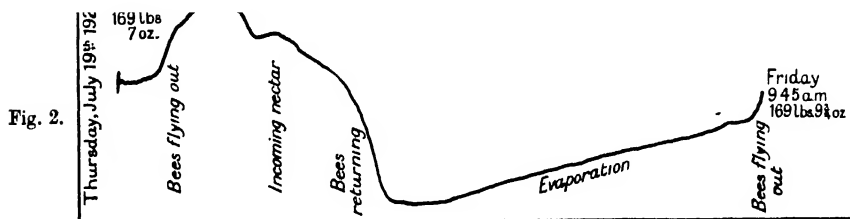
A device for automatically adding a weight has greatly extended the usefulness of this balance. A similar arrangement had previously been used by Oden<sup>(2)</sup> and Keen<sup>(1)</sup>. When the hive increases in weight, say during a honey flow, the pen reaches the bottom of the chart and touches the contact wire *C*, completing an electric circuit. The mechanism consists of an ordinary electric bell from which the gong has been removed. A saw cut has been made in the hammer and the knife *K* (a safety razor blade) inserted. The weight *W* (a bag of shot) is suspended by a thread which passes within a fraction of an inch of the blade without actually touching it. When the circuit is completed the knife saws rapidly through the thread. The weight then gently drops on to the pan, the beam returns to the level position and brings the pen to the centre of the chart, ready to record a further increase. If necessary it would be possible to arrange for the addition of further weights in this manner, but one bag of four pounds weight has been found to give sufficient margin to allow the machine to be left unattended on Sundays and holidays.

A typical daily chart during the honey flow is shown in Fig. 2. (It will be seen that the record made by this instrument is inverted: a rise in weight being indicated by a downward line.) In the morning there is at first a drop in weight due to the exodus of bees. Later the hive is for a time in equilibrium, the outgoing bees being balanced by those that are already returning loaded. Then there begins a rise; the full field force is now at work and arrivals and departures are about equal in numbers, but the arrivals bring nectar water or pollen, and the outgoing bees are not laden. This state of affairs continues while forage is available. Up to the present, no very marked instance of plants yielding nectar in the morning or in the afternoon only, has been noticed in this district. Irregularities are chiefly due to threatened storms or to the sky becoming overcast. In the evening bees are returning and fewer are going out and there is often a tendency for the curve to become steeper at this time. When the last foragers are home the loss of weight due to evaporation and respiration becomes evident. This has been masked during the day by the changes due to other activities of the hive. Evaporation continues steadily through the night until bees begin to fly on the following morning.

Fig. 3 is a similar chart and shows a case where the automatic weight release has come into play, allowing the trace to continue on the same chart without the intervention of the observer.

Fig. 4 is a composite chart in which the Monday curve is made to follow on from that of Sunday. At 6.45 p.m. the weight was set by the observer. The evaporation of surplus moisture on the night of July 8th-9th is well

seen. There is quite a small drop which may perhaps be attributed to the earliest scouts leaving the hive. Normally this is followed by a general exodus as in the two previous examples. However, on three or more consecutive nights at about this time, a definite rise was observed (marked with an asterisk), which appears to be due to the return of bees which had been benighted on the previous evening and taken shelter in the fields.



The moon was in the third quarter, the nights were warm and the bees were working white clover. It will be noticed that bees were working late on the previous night.

Many other facts are recorded on the tell-tale chart; as, for instance, when a mouse took its lodging among the quilts of the hive. On one occasion, after the honey flow was over, an unexpected increase in weight led to the detection of robbing from a neighbouring apiary. Wind causes much swaying even in the hut in which the instrument is housed. Fog

has sometimes been observed to cause an increase in weight during winter when no flying is taking place, owing to absorption of moisture by the combs and the wood of the hive. Distinct drops in weight during winter occur on those days when cleansing flights are taken, and these show a very definite relationship with the hours of sunshine recorded.

It has been suggested that some races of bees are early risers; that some work in unfavourable weather, while others are fair-weather bees, and that some work at certain sources of nectar neglected by others. Hives on recording scales, such as the one described, would be of great use in investigating these and other problems.

#### REFERENCES.

- (1) COUTTS, J. R. H., CROWTHER, E. M., KEEN, B. A. and ODEN, S. (1924). An Automatic and Continuous Recording Balance. *Proc. Roy. Soc. A*, cvI, 33-51.
- (2) ODEN, SVEN (1915-16). On the Size of the Particles in Deep Sea Deposits. *Proc. Roy. Soc. Edinburgh*, xxxvi (3), No. 13, 219-236.
- (3) PARKS, H. B. (1927). The Use of a Weight Scale in the Apiary. *Report of the State Apiarist, Iowa*, pp. 30-35.
- (4) WILLIAMS, C. B. (1927). A Chart Recording Weighing Machine for Beekeeping or other Research. *Bull. Ent. Research*, xviii (1), 63-65 (Pl. IV).

(Received November 29th, 1928.)



# A SURVEY OF THE INSECT AND OTHER INVERTEBRATE FAUNA OF PERMANENT PASTURE AND ARABLE LAND OF CERTAIN SOIL TYPES AT ABERYSTWYTH

By E. E. EDWARDS, M.Sc.

(*Department of Zoology, University College of Wales, Aberystwyth.*)

(With 4 Text-figures.)

## CONTENTS.

	PAGE
1. INTRODUCTION . . . . .	299
2. GENERAL DESCRIPTION OF THE DISTRICT . . . . .	300
3. DESCRIPTION OF AREAS EXAMINED . . . . .	301
(a) Pasture areas . . . . .	301
(b) Arable area . . . . .	303
4. BOTANICAL CENSUS OF THE AREAS . . . . .	303
(a) Method of investigation . . . . .	303
(b) Deductions from botanical analysis . . . . .	303
5. SOIL ANALYSIS OF THE AREAS EXAMINED . . . . .	305
(a) Mechanical analysis . . . . .	305
(b) Deductions from the mechanical analysis of the soils . . . . .	306
(c) Deductions from the chemical analysis of the soils . . . . .	307
6. METHOD OF INVESTIGATION OF FAUNA . . . . .	307
7. FAUNAL CENSUS OF INVESTIGATED AREAS . . . . .	309
8. DISCUSSION OF DATA FURNISHED BY FAUNAL CENSUS . . . . .	313
A. Total fauna:	
(a) In relation to the different soil types . . . . .	313
(b) In relation to depth . . . . .	316
B. Components of fauna in relation to the different soil types . . . . .	319
9. NOTE ON <i>OLIGOTROPHUS ALOPECURI</i> AND <i>APHIOCHAETAE BREVICOSTALIS</i> . . . . .	320
10. SUMMARY . . . . .	321
REFERENCES . . . . .	322

## 1. INTRODUCTION.

THE investigation described in this paper was carried out from January 1925 to February 1926. The fauna of four distinct soil types of a permanent pasture and one of an arable area has been considered. In order

## 302 *Invertebrate Fauna of Pasture and Arable Land*

sufficient to modify the heavy nature induced by the high proportion of clay and silt (see Table III under heading "Loss on ignition").

*Lighter Drift Area.* This consists of a narrow portion of about four acres on slightly higher ground than the alluvial area. Owing to its position, and the high percentage of the coarser particles, the soil is of a light open nature, and possesses an excellent drainage. It emerges indefinitely into the alluvial pasture below and, like it, has been under permanent pasture for many years. It has accumulated a large amount of organic matter, though naturally at a much slower rate than the low-lying alluvial soil.

**CAE MAWR.** The character of the soil in this field varies to a marked extent; that of the north-eastern portion contains a high proportion of clay and has been classified as a Boulder Clay area, that of the south-eastern portion is more sandy, whilst the area on the western side is a Sedentary soil.

Seeing that this field afforded at least two distinct soil types, a Boulder Clay area and a Sedentary soil area, and that the whole field had received practically the same treatment for a very long period, a difference in fauna might be expected to be due largely to the physical nature of the soil types.

*Boulder Clay Area.* Comprises about two acres of the north-east section of this field. On account of its tenacious character, with an underlying stratum of a heavy nature, which prevents leaching, it has a large retentive capacity for moisture and tends to become water-logged during wet weather. The disadvantage is not, however, very great owing to the drainage afforded by the slight slope towards the south and south-west, where it merges indefinitely into the Lighter Drift soil.

*Sedentary Area.* This narrow strip of four acres, lying to the west of the Boulder Clay area, separated from the latter by Lighter Drift soil and a hollow, down which flows a little stream and to which the land slopes sharply, contains soil of a Sedentary character. The latter is not very deep, but having a north-easterly aspect it is not very liable to suffer from drought.

Although the mechanical analysis of this soil (see Table III) shows it to contain a rather high proportion of clay and silt, yet the soil is of a light nature because it contains a high percentage of stones, especially so in the subsoil (see Table II).

Fringing this area on the western side is a narrow belt of wood, the latter being made up mainly of ash, oak and larch trees.

The distinction between the Sedentary soil and the Drift soils can be

easily recognised owing to the difference in both appearance and distribution of the stones.

(b) ARABLE AREA.

CAE GAT GOCH. The field has been continuously under the plough, apart from occasional rests in grass of from two to three years, and as a consequence contains an abnormally low amount of organic matter (see Table III, Sample 1).

It has a slight slope from the north side down to the south, but this does not amount to more than a few feet. It is of the Lighter Drift soil type, and it is only the road that separates this field from the Glacial Drift soil area of Cae'r Efail, which is of a similar origin and also resembles it in its mechanical composition (see Table III, Samples 13 and 1).

Thus the two fields afford excellent areas for comparison of the soil animal fauna. The difference in the constitution of the latter may be associated with cultural operations.

For the last two years the area was down under oats, and at the time of the investigation the land appeared in a clean condition.

#### 4. BOTANICAL CENSUS OF THE AREAS.

(a) METHOD OF INVESTIGATION.

The method of procedure consisted in cutting turf samples of a standard size. Each turf sample measured six inches square and five random samples were taken within an area of 10 by 15 yards, near where the samples for the soil population had been taken in each of the pasture fields under consideration. In this way a representative sample was obtained of the botanical nature of each area examined for its soil animal fauna. Both the number of plant species and their relative abundance, per five samples of each area investigated, is given in Table I.

(b) DEDUCTIONS FROM BOTANICAL ANALYSIS.

It will be noted that the Alluvial and Lighter Drift areas are good soil as indicated by the presence of *Alopecurus pratensis* in fairly large numbers, though in the Alluvial area the number of plants is probably lower than it might otherwise have been owing to conditions.

The Lighter Drift area is the best grazing agricultural sward, as it contains the least number of weeds, and the better grass species are present in fairly large numbers.

# 304 *Invertebrate Fauna of Pasture and Arable Land*

Table I.  
*Botanical Census (dominant species only).*

	Cae'r Efail		Cae Mawr	
	Alluvial	Lighter Drift	Boulder Clay	Sedentary
Gramineae:				
<i>Lolium perenne</i>	—	85	95	85
<i>Dactylis glomerata</i>	—	34	53	27
<i>Cynosurus cristatus</i>	—	—	234	368
<i>Alopecurus pratensis</i>	57	388	8	4
<i>Poa trivialis</i>	430	413	460	225
<i>Festuca rubra</i>	53	106	—	10
<i>Anthoxanthum odoratum</i>	36	—	—	35
Leguminosae:				
<i>Trifolium repens</i> *	10	4	109	190
Other orders (regarded as weeds):				
<i>Holcus lanatus</i>	160	213	347	390
<i>Agrostis alba</i>	28	55	149	103
<i>Ranunculus repens</i>	6	5	6	16
<i>Juncus effusus</i>	79	—	—	—
<i>Geranium dissectum</i>	22	—	10	8
<i>Carex</i> sp.	10	—	—	—
<i>Veronica officinalis</i>	20	—	4	14
<i>Bellis perennis</i>	—	—	8	37
<i>Leontodon autumnale</i>	—	—	2	14
Moss	Abundant	—	Common	Very common

\* Of the 190 plants in Sedentary area, three were *Trifolium pratense*.

The presence of *Juncus* and *Carex* species in the Alluvial area indicates a marshy condition and the large number of weed species, *e.g.* *Geranium* and *Veronica* species, suggests a tendency for open spaces.

The absence of *Lolium perenne*, *Dactylis glomerata* and *Cynosurus cristatus* in the Alluvial area is a further indication of poor sward; their absence being due to the marshy nature of the soil.

The botanical analysis composition of the Boulder Clay and Sedentary indicates a very dense sward of "low plants." There are a large number of species of grasses accompanied by a high number of plants. These areas contain also large quantities of *Trifolium repens*, suggesting that these soils are rich in nitrogen.

## 5. SOIL ANALYSIS OF THE AREAS EXAMINED.

## (a) MECHANICAL ANALYSIS.

Table II.

*Stones and Coarse Gravel in various Types of Soil.*

Type of soil	Ref. no. of sample	Soil			Subsoil		
		% stones above 1 cm. diam.	% coarse gravel 3 mm.- 1 cm. diam.	Total	% stones above 1 cm. diam.	% coarse gravel 3 mm.- 1 cm. diam.	Total
Alluvial	(3) Cae'r Efail	3.0	79.2	82.2	1.5	40.3	41.8
Lighter drift	(1) Gat Goch	29.3	30.9	60.2	112.0	66.0	178.0
Boulder clay	(11) Cae Mawr	10.4	40.0	50.4	0.5	50.0	50.5
Sedentary	(4) Bank	73.5	85.0	158.5	112.0	98.0	210.0

The above percentages have been calculated from the amount of air-dried soil passing through a 3 mm. sieve.

Table III.

*Mechanical Analysis of Nantcellan Soils.*

Type of soil	Alluvial	Lighter Drift		Boulder Clay	Sedentary
		Cae'r Efail	Gat Goch		
Name of field sample	Cae'r Efail (3)	Cae'r Efail (13)	Gat Goch (1)	Cae Mawr (17)	Cae Mawr (18)
Fine gravel ...	1.3	15.3	19.8	1.0	12.4
Coarse sand ...	1.3	11.4	9.5	2.2	7.6
Fine sand ...	3.9	10.1	11.0	12.8	16.9
Silt ...	12.5	15.3	11.5	14.4	12.2
Fine silt ...	33.3	29.0	26.9	35.7	24.0
Clay ...	21.5	7.1	9.1	15.4	11.0
Moisture ...	6.5	2.8	3.0	2.5	2.3
Loss on ignition	22.9	11.4	9.5	14.7	13.1
CaCO <sub>3</sub> ...	Nil	0.01	0.02	—	—
<i>Subsoil.</i>					
Fine gravel ...	0.0	20.9	33.2	0.0	14.4
Coarse sand ...	0.6	14.6	13.7	0.3	7.8
Fine sand ...	13.5	9.8	10.8	9.2	17.1
Silt ...	12.8	11.7	8.7	15.3	10.6
Fine silt ...	34.7	24.8	18.0	35.5	26.9
Clay ...	21.3	7.1	9.2	28.2	10.9
Moisture ...	3.7	2.3	2.8	1.6	1.7
Loss on ignition	11.1	8.5	6.1	9.8	8.9
CaCO <sub>3</sub> ...	—	—	—	—	—

## (b) DEDUCTIONS FROM THE MECHANICAL ANALYSIS OF THE SOILS.

It will be noted that the relative proportions of the total mineral ingredients of both the soil and subsoil of the two areas of the Glacial Lighter Drift soil formation are similar. These two areas are under similar environmental conditions, except that Cae'r Efail area is a permanent pasture and Gat Goch arable land, suggesting the possibility of expressing the difference in the soil fauna in terms of cultural operations.

There is in the Alluvial and Boulder Clay areas an exceptionally high percentage of fine silt accompanied by a high proportion of clay, which gives the soil its characteristic plastic nature. The amount of fine silt in the two areas is practically equal, but the total amount of clay is much greater in the Alluvial area. The percentage of fine gravel and coarse sand in both samples is very low, and does not increase in proportion on passing from soil to subsoil.

The relative proportion of clay and fine silt in the soil is important, not only in that it hinders the rapid movement of soil inhabitants, but that it also determines the amount of gravitational water, which is very often the detrimental factor of soil life. The finer the soil particles the greater the pore space, and consequently the greater the quantity of water retained. Also, the slower the percolation of water through such soil, the greater the capillary capacity will be; thus the soil becomes waterlogged after heavy rainfalls. Under the latter conditions all soil air is expelled and the water therein will contain less dissolved air or oxygen and the rapid diffusion of soil air in all directions will be lessened owing to lowering of the temperature. So both the animals and the plants will suffer, the former due to the absence of free air, the latter to deficiency of dissolved air.

The mechanical analysis composition of the Heavier Drift soil indicates a Boulder Clay formation, but the high amount of organic matter, the natural slope of the land facilitating drainage, makes it more or less of the nature of a lighter type of soil.

The Alluvial soil has a mineral composition similar to that of the Boulder Clay soil, but has a much higher percentage of organic matter. Owing to the low situation of the field, and the absence of sufficient drainage, the high amount of organic matter by no means obliterates the harmful effect of the fine silt and clay on the texture of the soil. It would thus appear that the difference in the fauna of these two soil types may be due mainly to the high water content of the Alluvial soil.

It will be further noted that the Lighter Drift soil contains a much

higher percentage of fine gravel and coarse sand than the Boulder Clay soil; thus an attempt may be made to relate faunistic differences to a difference in size of soil particles.

For an examination of the mechanical analysis of the fine earth of the sedentary soil (Table III), one would regard it as of a heavy nature, but as it contains such a high proportion of stones (Table II, sample 4) and rather a high percentage of organic matter (Table III, sample 18), its heavy nature is modified to such an extent that it has some of the characteristics of a light soil. This area resembles in its mechanical formation that of the Lighter Drift soil area of Cae'r Efail, thus suggesting an attempt to relate faunistic differences to differences in stone content, the latter being in turn related to geological differences.

#### (c) DEDUCTIONS FROM THE CHEMICAL ANALYSIS OF THE SOILS.

Chemical analyses were carried out and it was found that there is a remarkable similarity in the total amounts of mineral ingredients present in different types of soil. As a consequence, the possibility that any of the essential mineral ingredients necessary for the natural growth of plants might be a determining factor of importance in relation to faunal differences between the investigated areas is reduced to a minimum.

The amount of lime present is negligible but the reactions of the majority of the soils indicate that no marked sourness has developed and no bad effects on the herbage could be traced. Undoubtedly, more liming would have an appreciable effect on the fertility of the Boulder Clay, as it would ameliorate the unfavourable physical properties caused by the high proportion of clay.

#### 6. METHOD OF INVESTIGATION OF FAUNA.

The method of taking the sample was in the main the same as that employed by Morris, except that the lower six inches was taken, in two samples of three inches each. The deeper samples are thus comparable with those of Thompson (1924) rather than those of Morris (1922) who divided his lower six inches into three samples of two inches each.

The soil was removed in this way to a depth of nine inches, giving four samples, which consisted of: I, the soil between the surface and a depth of one inch; II, the soil between a depth of one inch and a depth of three inches; III, the soil between three inches and six inches; IV, the soil between six inches and nine inches. The samples thus obtained were taken to the laboratory and examined. By taking a small quantity of

soil at a time, and examining it carefully, it was possible to obtain most of the insects and their larvae, Nematoda, Annelida and Mollusca, except Coleopterous adults feigning death, a few larvae of Coleoptera and Diptera and most Collembola and Acarina.

After this preliminary sifting, a portion at a time of the soil was taken and washed through a series of three sieves each eighteen inches in diameter, with meshes of 3.5 mm., 1.5 mm. and fifty meshes to the linear inch, respectively. The mesh of final sieve was the same as that used by Thompson, and was small enough to retain all the insects and their larvae, and the Acarina that were not detected by the preliminary sorting. The sieves at the same time were shaken to allow the finest soil particles to pass through the meshes. When all the sediment had passed through, both residue and filtrate were examined. No organisms were ever found in the filtrate, so after the preliminary experiments the filtrate was neglected.

The residue on each sieve was then washed out separately into a shallow dish, and water added until the dish was half full. As the clay had all been washed out, the water remained clear, and by gently stirring the residue the organisms between the soil particles were released, and either floated on the surface of the water or remained on the surface of the residue at the bottom of the dish. The process was repeated until nothing further was found; usually one stirring was sufficient in the case of the residue retained by the uppermost two sieves. Finally the actual solid residue was examined but very rarely was anything present except a few small Oligochaeta and Nematoda.

In most cases, the larvae and pupae of insects could not be specifically identified and it was only by breeding out of the adult forms that they could be determined with exactness. At the same time, when plenty of material was at hand, some of them were preserved in 4 per cent. formalin or mounted on slides. All the adults reared were either set or preserved in formalin or 75 per cent. alcohol, along with, in most cases, one or more larvae and pupae of their particular species. When success did not attend the rearing of a species, it was nevertheless possible in most cases to indicate its family and sometimes also its genus.

The investigation is intended more to illustrate the differences between the different types of soil than seasonal variation.

Four samples were examined from each of the five soil types and were taken in January 1925, July, October, and January 1926 respectively. The samples of the different soils taken in any given month were taken at dates as close together as possible. Care was taken in selecting the



samples to avoid patches influenced by any special factors, such as proximity of hedges, etc.

I express my thanks for help in the identification of species of Insecta, Myriapoda and Mollusca to Messrs S. G. Brade-Birks, G. C. Robson, G. H. Carpenter, F. W. Edwards, R. Stenton, and B. S. Williams.

#### 7. FAUNAL CENSUS OF INVESTIGATED AREAS.

In the following list the Oligochaeta have been divided into two groups: (a) Terricolae, which includes such forms as *Lumbricus terrestris*, and (b) Limicolae, which includes the small white forms. The formula used is based on that used by Morris (1922). The numbers outside the brackets give the number of the sample<sup>1</sup> (or samples) in which the species occurred and the letters the areas in which they were found, A. = Alluvial pasture, B. = Lighter Drift pasture, C. = Lighter Drift arable, D. = Boulder Clay pasture, E. = Sedentary pasture. The first numbers within the brackets give, above, the total number found, and below, in Roman numerals, the layer (or layers) in which they were found. The second numbers within brackets give, above, the greatest number found at any one level, and below, in Roman numerals, the level at which they were found. The letter (or letters) in brackets placed after the name of a species indicate the stage of development, i.e. (L.)—Larval; (P.)—Pupal; (A.)—Adult.

#### *Species common in all Areas.*

\* Indicates that insects have been reared by the writer from larvae found in species thus marked.

**Nematoda.** Spp. A. 1, 3  $\left( \begin{smallmatrix} 6 \\ \text{I, II} \end{smallmatrix}; \begin{smallmatrix} 4 \\ \text{I} \end{smallmatrix} \right)$ ; B. 1, 3, 4  $\left( \begin{smallmatrix} 45 \\ \text{I-IV} \end{smallmatrix}; \begin{smallmatrix} 18 \\ \text{III} \end{smallmatrix} \right)$ ; C. 1, 3, 4  $\left( \begin{smallmatrix} 28 \\ \text{I-IV} \end{smallmatrix}; \begin{smallmatrix} 9 \\ \text{III} \end{smallmatrix} \right)$ ; D. 1, 3, 4  $\left( \begin{smallmatrix} 8 \\ \text{II-IV} \end{smallmatrix}; \begin{smallmatrix} 3 \\ \text{IV} \end{smallmatrix} \right)$ ; E. 1-4  $\left( \begin{smallmatrix} 66 \\ \text{I-IV} \end{smallmatrix}; \begin{smallmatrix} 13 \\ \text{IV} \end{smallmatrix} \right)$ .

**Oligochaeta.** (TERRICOLAE.) A. 1-4  $\left( \begin{smallmatrix} 94 \\ \text{I-III} \end{smallmatrix}; \begin{smallmatrix} 48 \\ \text{I} \end{smallmatrix} \right)$ ; B. 1-4  $\left( \begin{smallmatrix} 78 \\ \text{I-IV} \end{smallmatrix}; \begin{smallmatrix} 19 \\ \text{I} \end{smallmatrix} \right)$ ; C. 1-4  $\left( \begin{smallmatrix} 31 \\ \text{I-IV} \end{smallmatrix}; \begin{smallmatrix} 6 \\ \text{IV} \end{smallmatrix} \right)$ ; D. 1-4  $\left( \begin{smallmatrix} 116 \\ \text{I-IV} \end{smallmatrix}; \begin{smallmatrix} 33 \\ \text{I} \end{smallmatrix} \right)$ ; E. 1-4  $\left( \begin{smallmatrix} 89 \\ \text{I-IV} \end{smallmatrix}; \begin{smallmatrix} 19 \\ \text{I} \end{smallmatrix} \right)$ .

(TERRICOLAE.) Cocoons. A. 1, 3, 4  $\left( \begin{smallmatrix} 36 \\ \text{I, II} \end{smallmatrix}; \begin{smallmatrix} 18 \\ \text{I} \end{smallmatrix} \right)$ ; B. 1-4  $\left( \begin{smallmatrix} 63 \\ \text{I-IV} \end{smallmatrix}; \begin{smallmatrix} 14 \\ \text{II} \end{smallmatrix} \right)$ ; C. 1, 3, 4  $\left( \begin{smallmatrix} 26 \\ \text{II-IV} \end{smallmatrix}; \begin{smallmatrix} 4 \\ \text{II} \end{smallmatrix} \right)$ ; D. 1-4  $\left( \begin{smallmatrix} 112 \\ \text{I-IV} \end{smallmatrix}; \begin{smallmatrix} 65 \\ \text{I} \end{smallmatrix} \right)$ ; E. 1-4  $\left( \begin{smallmatrix} 130 \\ \text{I-IV} \end{smallmatrix}; \begin{smallmatrix} 58 \\ \text{I} \end{smallmatrix} \right)$ .

(LIMICOLAE.) A. 1-4  $\left( \begin{smallmatrix} 344 \\ \text{I, II} \end{smallmatrix}; \begin{smallmatrix} 135 \\ \text{I} \end{smallmatrix} \right)$ ; B. 1-4  $\left( \begin{smallmatrix} 292 \\ \text{I-IV} \end{smallmatrix}; \begin{smallmatrix} 77 \\ \text{I} \end{smallmatrix} \right)$ ; C. 1-4  $\left( \begin{smallmatrix} 83 \\ \text{II-IV} \end{smallmatrix}; \begin{smallmatrix} 6 \\ \text{III} \end{smallmatrix} \right)$ ; D. 1-4  $\left( \begin{smallmatrix} 385 \\ \text{I-IV} \end{smallmatrix}; \begin{smallmatrix} 106 \\ \text{I} \end{smallmatrix} \right)$ ; E. 1-4  $\left( \begin{smallmatrix} 355 \\ \text{I-IV} \end{smallmatrix}; \begin{smallmatrix} 72 \\ \text{I} \end{smallmatrix} \right)$ .

<sup>1</sup> I.e. 1 = sample taken in January 1925; 2 = in July; 3 = in October; 4 = in January 1926.

## 310 *Invertebrate Fauna of Pasture and Arable Land*

**ARACHNIDA. Araneida.** Spp. A. 3, 4  $\left(\frac{3}{I}\right)$ ; B. 2, 3  $\left(\frac{5}{I}\right)$ ; C. 3, 4  $\left(\frac{2}{I}\right)$ ; D. 2-4  $\left(\frac{6}{I}\right)$ ; E. 2-4  $\left(\frac{6}{I}\right)$ .

**Acarina.** Spp. A. 1-4  $\left(\frac{228}{I, II}; \frac{102}{I}\right)$ ; B. 1-4  $\left(\frac{178}{I, II, IV}; \frac{60}{I}\right)$ ; C. 1-4  $\left(\frac{59}{I-III}; \frac{13}{I}\right)$ ; D. 1-4  $\left(\frac{95}{I-IV}; \frac{46}{I}\right)$ ; E. 1-4  $\left(\frac{128}{I, II}; \frac{50}{I}\right)$ .

**INSECTA. Collembola.** *Onychiurus armatus* (Tulb.) A. 2-4  $\left(\frac{30}{I-IV}; \frac{12}{III}\right)$ ; B. 1-4  $\left(\frac{899}{I-IV}; \frac{160}{I}\right)$ ; C. 1-4  $\left(\frac{540}{I-IV}; \frac{77}{II}\right)$ ; D. 1-4  $\left(\frac{85}{I-IV}; \frac{46}{I}\right)$ ; E. 1-4  $\left(\frac{42}{I-IV}; \frac{12}{I}\right)$ ; *Achorutes armatus* (Nic.) A. 4  $\left(\frac{4}{I}\right)$ ; B. 1-4  $\left(\frac{87}{I-IV}; \frac{21}{I}\right)$ ; C. 4  $\left(\frac{41}{I-IV}; \frac{15}{I}\right)$ ; D. 1-4  $\left(\frac{130}{I, II}; \frac{51}{I}\right)$ ; E. 1-4  $\left(\frac{125}{I-IV}; \frac{60}{I}\right)$ ; *Isotoma viridis* Bourl. A. 2-4  $\left(\frac{43}{I}; \frac{25}{I}\right)$ ; B. 1-4  $\left(\frac{40}{I, II}; \frac{15}{I}\right)$ ; C. 1, 3  $\left(\frac{7}{I, II}\right)$ ; D. 1-4  $\left(\frac{20}{I, II}; \frac{8}{I}\right)$ ; E. 1-4  $\left(\frac{40}{I, II}; \frac{21}{I}\right)$ ; *Isotomurus palustris* (Müll.) A. 3-4  $\left(\frac{22}{I-III}; \frac{13}{I}\right)$ ; B. 3, 4  $\left(\frac{2}{I, IV}\right)$ ; C. 3  $\left(\frac{1}{I}\right)$ ; D. 1-4  $\left(\frac{55}{I-IV}; \frac{19}{I}\right)$ ; E. 1, 3  $\left(\frac{13}{I-III}; \frac{7}{I}\right)$ .

Immature forms of the following families also occurred:

**ONYCHIURIDAE.** A. 4  $\left(\frac{4}{III}\right)$ ; B. 2, 3  $\left(\frac{5}{I-III}\right)$ ; C. 3  $\left(\frac{21}{I}\right)$ ; D. 2, 4  $\left(\frac{24}{I-IV}; \frac{12}{I}\right)$ ; E. 2  $\left(\frac{4}{III, IV}\right)$ ; **ISOTOMIDAE.** A. 3, 4  $\left(\frac{17}{I}; \frac{10}{I}\right)$ ; B. 3, 4  $\left(\frac{7}{I}; \frac{4}{I}\right)$ ; C. 1-4  $\left(\frac{7}{I, II}; \frac{2}{I}\right)$ ; D. 2, 3  $\left(\frac{5}{I}; \frac{3}{I}\right)$ ; E. 3  $\left(\frac{1}{I}\right)$ .

**Coleoptera.** *Atheta analis* A. (A). A. 3, 4  $\left(\frac{3}{I}\right)$ ; B. 2, 3  $\left(\frac{3}{I, II}\right)$ ; C. 3  $\left(\frac{1}{I}\right)$ ; D. 1, 2, 4  $\left(\frac{3}{I}\right)$ ; E. 3, 4  $\left(\frac{3}{I}\right)$ .

**Diptera.** *Limosina sylvatica* Mg.\* A. 3, 4  $\left(\frac{2}{I}\right)$ ; B. 3  $\left(\frac{3}{I}\right)$ ; C. 1, 4  $\left(\frac{9}{II, III}; \frac{4}{III}\right)$ ; D. 1, 3, 4  $\left(\frac{9}{I, II}; \frac{4}{I}\right)$ ; E. 1, 3, 4  $\left(\frac{7}{I}\right)$ .

### *Species occurring in from two to four Areas.*

**GASTROPODA.** *Hyalinia radiatula* Alder. A. 1, 3, 4  $\left(\frac{6}{I}; \frac{2}{I}\right)$ ; B. 1, 4  $\left(\frac{2}{I}\right)$ ; E. 3  $\left(\frac{1}{I}\right)$ ; *Vallonia pulchella* Müll. B. 1, 3, 4  $\left(\frac{10}{I}; \frac{5}{I}\right)$ ; E. 2-4  $\left(\frac{7}{I}; \frac{3}{I}\right)$ ; *Cochlicopa lubrica* Müll. A. 1, 3, 4  $\left(\frac{9}{I}; \frac{4}{I}\right)$ ; E. 3, 4  $\left(\frac{2}{I}\right)$ ; *Carychium minimum* Müll. A. 4  $\left(\frac{1}{I}\right)$ ; D. 1, 4  $\left(\frac{2}{I}\right)$ ; *Limax agrestis* (Linne) D. 3  $\left(\frac{2}{I}\right)$ ; E. 1  $\left(\frac{1}{I}\right)$ ; *Limax* spp. B. 1  $\left(\frac{3}{I}\right)$ ; C. 1, 3  $\left(\frac{2}{I}\right)$ ; D. 4  $\left(\frac{3}{I}\right)$ ; E. 3, 4  $\left(\frac{3}{I}\right)$ .

**MYRIAPODA.** *Brachyiulus* (*Microbrachyiulus*) *pusillus* (Leach) C. 1-4  $\left(\frac{34}{I-IV}; \frac{4}{I}\right)$ ; D. 3  $\left(\frac{1}{III}\right)$ ; *Geophilus longicornis* Leach B. 1  $\left(\frac{3}{II, IV}\right)$ ; C. 1-4  $\left(\frac{14}{I-IV}; \frac{3}{III}\right)$ ; D. 1  $\left(\frac{1}{I}\right)$ ;

E. 4  $\left(\frac{3}{\text{I, III}}; \frac{2}{\text{I}}\right)$ ; *Symphyla* spp. B. 1-4  $\left(\frac{58}{\text{I-IV}}; \frac{18}{\text{IV}}\right)$ ; C. 1-4  $\left(\frac{162}{\text{I-IV}}; \frac{61}{\text{IV}}\right)$ ; D. 1-4  $\left(\frac{39}{\text{III, IV}}; \frac{17}{\text{IV}}\right)$ ; E. 1-4  $\left(\frac{55}{\text{II-IV}}; \frac{21}{\text{IV}}\right)$ .

**INSECTA. Collembola.** *Onychiurus fimetarius* (Linn.) A. 3  $\left(\frac{5}{\text{I}}\right)$ ; B. 1, 3  $\left(\frac{10}{\text{I, II}}; \frac{6}{\text{I}}\right)$ ; C. 3  $\left(\frac{2}{\text{I}}\right)$ ; *Tullbergia quadrispina* (Börn.) B. 1-4  $\left(\frac{224}{\text{II-IV}}; \frac{41}{\text{III}}\right)$ ; C. 1-4  $\left(\frac{998}{\text{I-IV}}; \frac{150}{\text{III}}\right)$ ; D. 1-4  $\left(\frac{394}{\text{I-IV}}; \frac{90}{\text{I}}\right)$ ; E. 1-4  $\left(\frac{584}{\text{I-IV}}; \frac{90}{\text{IV}}\right)$ ; *Folsomia fimetaria* (Linn.) B. 1-4  $\left(\frac{143}{\text{I-IV}}; \frac{41}{\text{II}}\right)$ ; C. 1-4  $\left(\frac{30}{\text{II, III}}; \frac{10}{\text{II}}\right)$ ; E. 1-4  $\left(\frac{95}{\text{I-IV}}; \frac{35}{\text{I}}\right)$ ; *Sminthurus viridis* Lubbo. B. 2  $\left(\frac{1}{\text{I}}\right)$ ; C. 3  $\left(\frac{5}{\text{I-III}}; \frac{2}{\text{I}}\right)$ ; D. 1  $\left(\frac{1}{\text{IV}}\right)$ .

**Thysanoptera.** Spp. B. 3  $\left(\frac{1}{\text{I}}\right)$ ; C. 3, 4  $\left(\frac{3}{\text{I-III}}\right)$ .

**Rhynchota.** *Aphis* spp. A. 3  $\left(\frac{1}{\text{I}}\right)$ ; B. 3  $\left(\frac{6}{\text{I}}\right)$ ; D. 3, 4  $\left(\frac{6}{\text{I}}\right)$ ; E. 2, 3  $\left(\frac{7}{\text{I}}\right)$ .

**Lepidoptera.** Spp. B. 3  $\left(\frac{1}{\text{II}}\right)$ ; C. 1  $\left(\frac{1}{\text{I}}\right)$ ; D. 3  $\left(\frac{1}{\text{I}}\right)$ ; E. 1, 4  $\left(\frac{3}{\text{I}}\right)$ .

**Coleoptera.** *Xantholinus longiventris* Heer. (A.) B. 4  $\left(\frac{1}{\text{I}}\right)$ ; C. 1, 4  $\left(\frac{2}{\text{I}}\right)$ ; D. 3  $\left(\frac{1}{\text{I}}\right)$ ; E. 3  $\left(\frac{2}{\text{I}}\right)$ ; *Philonthus nigrutilus* Grav. (A.) A. 4  $\left(\frac{2}{\text{I}}\right)$ ; D. 4  $\left(\frac{1}{\text{I}}\right)$ ; E. 1  $\left(\frac{1}{\text{I}}\right)$ ; *Stenus brunripes* Steph. (A.) B. 3  $\left(\frac{1}{\text{I}}\right)$ ; D. 1  $\left(\frac{1}{\text{I}}\right)$ ; *Tachyporus brunneus* Fab. (A.) B. 4  $\left(\frac{1}{\text{I}}\right)$ ; D. 1  $\left(\frac{1}{\text{I}}\right)$ ; E. 3  $\left(\frac{1}{\text{I}}\right)$ ; *Trichopteryx* spp. (A.) B. 1, 3  $\left(\frac{2}{\text{I}}\right)$ ; E. 1, 3  $\left(\frac{3}{\text{I}}\right)$ ; *Cantharis (Telo-phorus) rufa* Linn. var. *lituratus* Fall.\* (L.) A. 4  $\left(\frac{6}{\text{I}}\right)$ ; B. 4  $\left(\frac{4}{\text{I}}\right)$ ; E. 3  $\left(\frac{1}{\text{I}}\right)$ ; *Dryops (Parnus)* sp. (A.) A. 2  $\left(\frac{1}{\text{I}}\right)$ ; B. 2, 4  $\left(\frac{2}{\text{I}}\right)$ ; *Agriotes obscuris* L. (L.) A. 3  $\left(\frac{1}{\text{II}}\right)$ ; B. 1-4  $\left(\frac{3}{\text{I, II}}\right)$ ; D. 3, 4  $\left(\frac{5}{\text{I}}\right)$ ; E. 3  $\left(\frac{1}{\text{II}}\right)$ ; *Athous haemorrhoidalis* F. (L.) A. 3  $\left(\frac{1}{\text{II}}\right)$ ; D. 1-4  $\left(\frac{8}{\text{I-IV}}; \frac{2}{\text{III}}\right)$ . Unidentified larvae and pupae: STAPHYLINIDAE B. 4  $\left(\frac{1}{\text{I}}\right)$ ; C. 1-4  $\left(\frac{4}{\text{I-III}}\right)$ ; D. 2  $\left(\frac{5}{\text{I, IV}}; \frac{2}{\text{I}}\right)$ ; E. 3, 4  $\left(\frac{6}{\text{I, III}}; \frac{2}{\text{I}}\right)$ ; unclassified B. 2, 4  $\left(\frac{18}{\text{I, II}}; \frac{8}{\text{IV}}\right)$ ; C. 3, 4  $\left(\frac{2}{\text{I}}\right)$ ; D. 3  $\left(\frac{1}{\text{I}}\right)$ ; E. 2, 3  $\left(\frac{2}{\text{I}}\right)$ .

**Diptera.** *Oligotrophus alopecuri* Reut. (?)\* A. 1, 3, 4  $\left(\frac{245}{\text{I, II}}; \frac{235}{\text{I}}\right)$ ; E. 4  $\left(\frac{1}{\text{I}}\right)$ ; *Sciara annulata* Mg.\* B. 3, 4  $\left(\frac{2}{\text{I}}\right)$ ; D. 1-3  $\left(\frac{8}{\text{I}}\right)$ ; *Bibio johannis* L. (L.) A. 3  $\left(\frac{8}{\text{I}}\right)$ ; B. 4  $\left(\frac{10}{\text{I}}\right)$ ; C. 1, 4  $\left(\frac{7}{\text{I, III}}; \frac{6}{\text{III}}\right)$ ; E. 3, 4  $\left(\frac{36}{\text{I}}\right)$ ; *Tipula oleracea* L.\* B. 3  $\left(\frac{1}{\text{I}}\right)$ ; D. 1  $\left(\frac{2}{\text{I}}\right)$ ; E. 1  $\left(\frac{1}{\text{I}}\right)$ ; T. spp. (L.) B. 2, 4  $\left(\frac{4}{\text{I}}\right)$ ; C. 4  $\left(\frac{2}{\text{I}}\right)$ ; E. 3, 4  $\left(\frac{3}{\text{I}}\right)$ ; *Empis livida*\* A. 1-4  $\left(\frac{9}{\text{I}}\right)$ ; B. 3-4  $\left(\frac{5}{\text{I}}\right)$ ; E.  $\left(\frac{1}{\text{I}}\right)$ ; *Ramphomyia spinipes* Flin.\* B. 1, 3  $\left(\frac{12}{\text{I-III}}; \frac{8}{\text{II}}\right)$ ; D. 2, 4  $\left(\frac{3}{\text{I, II}}; \frac{2}{\text{I}}\right)$ ; E. 1, 3, 4

## 312 *Invertebrate Fauna of Pasture and Arable Land*

$\left(\frac{7}{\text{I-IV}}; \frac{5}{\text{I}}\right)$ ; *Chloromyia formosa* Scop.\* D. 3, 4  $\left(\frac{13}{\text{I}}\right)$ ; E. 4  $\left(\frac{1}{\text{I}}\right)$ ; *Coelopa pilipes* Hal.\* B. 1, 3  $\left(\frac{12}{\text{I-III}}; \frac{7}{\text{II}}\right)$ ; C. 1  $\left(\frac{1}{\text{III}}\right)$ ; D. 2, 4  $\left(\frac{2}{\text{I, II}}\right)$ ; E. 1, 3, 4  $\left(\frac{7}{\text{I-IV}}; \frac{4}{\text{I}}\right)$ ; *Polietes albineata* F. C. 1  $\left(\frac{6}{\text{II, III}}; \frac{5}{\text{III}}\right)$ ; E. 4  $\left(\frac{1}{\text{I}}\right)$ ; *Tanytus maculatus* (L.) A. 1  $\left(\frac{1}{\text{I}}\right)$ ; C. 1  $\left(\frac{1}{\text{I}}\right)$ ; D. 1, 2  $\left(\frac{3}{\text{I, II}}\right)$ ; E. 1  $\left(\frac{1}{\text{I}}\right)$ ; *Helobia* sp. (L.) A. 1, 4  $\left(\frac{33}{\text{I, II}}; \frac{24}{\text{I}}\right)$ ; E. 1  $\left(\frac{1}{\text{I}}\right)$ . Unidentified larvae and pupa: CECIDOMYIDAE B. 3  $\left(\frac{2}{\text{I}}\right)$ ; C. 3  $\left(\frac{1}{\text{I}}\right)$ ; D. 1-4  $\left(\frac{28}{\text{I}}\right)$ ; E. 3  $\left(\frac{9}{\text{I}}\right)$ ; MYCETOPHILIDAE D. 4  $\left(\frac{72}{\text{I-III}}; \frac{45}{\text{III}}\right)$ ; E. 4  $\left(\frac{31}{\text{I}}\right)$ ; unclassified (L.) A. 4  $\left(\frac{1}{\text{I}}\right)$ ; B. 4  $\left(\frac{15}{\text{I-III}}; \frac{8}{\text{I}}\right)$ ; D. 2, 3, 4  $\left(\frac{18}{\text{I}}\right)$ ; E. 3, 4  $\left(\frac{9}{\text{I}}\right)$ .

### *Species confined to a single Area.*

**MYRIAPODA.** *Blaniulus guttulatus* (Bosc.) C. 1-4  $\left(\frac{15}{\text{I-III}}; \frac{4}{\text{I}}\right)$ ; *Monotarsobius duboscqui* (Brolemann) C. 1-4  $\left(\frac{13}{\text{I-IV}}; \frac{2}{\text{I}}\right)$ ; *Geophilus insculptus* Attems. C. 3  $\left(\frac{2}{\text{I, IV}}\right)$ ; *Schendyla memorensis* (C. L. Koch) C. 1-4  $\left(\frac{4}{\text{II-IV}}\right)$ ; *Brachydesmus superus moscellanus* Verhoeff. C. 3  $\left(\frac{3}{\text{I, II}}; \frac{2}{\text{I}}\right)$ .

**INSECTA. Collembola.** *Schottella parvula* (Schäff.) C. 3  $\left(\frac{14}{\text{I}}\right)$ ; *Xenylla brevicauda* Tulb. B. 3  $\left(\frac{1}{\text{IV}}\right)$ ; *Folsomia quadrivulata* (Tulb.) A. 1-4  $\left(\frac{130}{\text{I-IV}}; \frac{57}{\text{I}}\right)$ ; *Sinella curviseta* Brook. D. 2  $\left(\frac{8}{\text{III, IV}}\right)$ ; *Sminthurus aureus* (Lubb.) B. 1, 3  $\left(\frac{2}{\text{I, III}}\right)$ ; *Bourletiella lutea* (Lubb.) C. 4  $\left(\frac{7}{\text{II}}\right)$ ; *Arrhopalites coecus* (Tulb.) B. 4  $\left(\frac{1}{\text{II}}\right)$ .

**Thysanura.** Sp. E. 2  $\left(\frac{1}{\text{I}}\right)$ .

**Rhynchota.** *Athysanus communis* J. Sahl. B. 3  $\left(\frac{1}{\text{I}}\right)$ .

**Coleoptera.** *Nebria brevicollis* F. (A.) C. 3  $\left(\frac{1}{\text{I}}\right)$ ; *Pterostichus vernalis* Gyll. (A.) E. 4  $\left(\frac{1}{\text{I}}\right)$ ; *Notiophilus substriatus* Wat. (A.) C. 3  $\left(\frac{1}{\text{I}}\right)$ ; *Bembidium obtusum* Sturm. (A.) D. 2  $\left(\frac{1}{\text{I}}\right)$ ; CARABIDAE sp. (L.) E. 1  $\left(\frac{1}{\text{I}}\right)$ ; *Xantholinus linearis* Ol. (A.) E. 4  $\left(\frac{2}{\text{I}}\right)$ ; *X. punctulatus* (Payk.) (A.) B. 1  $\left(\frac{1}{\text{I}}\right)$ ; *Quedius boops* Grav. (A.) D. 3  $\left(\frac{1}{\text{I}}\right)$ ; *Othius laeviusculus* Steph. (A.) D. 3  $\left(\frac{1}{\text{I}}\right)$ ; *Philonthus politus* F. (A.) E. 3  $\left(\frac{1}{\text{I}}\right)$ ; *P. umbratilis* Grav. (A.) D. 1  $\left(\frac{1}{\text{I}}\right)$ ; *P. varius* Gyll. (A.) E. 1  $\left(\frac{1}{\text{I}}\right)$ ; *Platystethus arenarius* Fourc. (A.) B. 4  $\left(\frac{1}{\text{I}}\right)$ ; *Oxytelus tetracarinatus* (Block) (A.) A. 2  $\left(\frac{1}{\text{IV}}\right)$ ; *O. sculpturatus* Grav. (A.) D. 4  $\left(\frac{1}{\text{I}}\right)$ ; *Stenus declaratus* Er. (A.)

E. 1  $\left(\frac{1}{I}\right)$ ; *Tachyporus obtusus* L. (A.) B. 2  $\left(\frac{1}{I}\right)$ ; *Mycetoporus splendidulus* (A.) D. 3  $\left(\frac{1}{I}\right)$ ; *Tachinus laticollis* Grav. (A.) C. 2  $\left(\frac{1}{I}\right)$ ; *Helophorus aeneipennis* Thoms. (A.) A. 3  $\left(\frac{1}{I}\right)$ ; *Cercyon melanocephalus* L. (A.) E. 3  $\left(\frac{1}{I}\right)$ ; *C. lateralis* Marsh. (A.) D. 3  $\left(\frac{1}{I}\right)$ ; *Megasternum boletophagum* Marsh. (A.) D. 4  $\left(\frac{1}{I}\right)$ ; *Cartoderc filum* Aube. (A.) E. 2  $\left(\frac{1}{IV}\right)$ ; *Plinus lectus* F. (A.) B. 1  $\left(\frac{1}{IV}\right)$ . *Aphodius fimetarius* L. (A.) B. 3  $\left(\frac{1}{I}\right)$ ; *A. punctatosulcatus* Sturm. (A.) E. 1  $\left(\frac{1}{I}\right)$ ; *Agriotes sputator* (?) (L.) D. 4  $\left(\frac{1}{II}\right)$ ; *Sitones lineatus* (A.) C. 1-3  $\left(\frac{3}{I}\right)$ ; *Liosoma ovatum* Clairv. (A.) D. 4  $\left(\frac{1}{I}\right)$ ; *Barynotus obscurus* (A.) D. 1  $\left(\frac{1}{I}\right)$ .

**Hymenoptera.** *Cynipidae* sp. (A.) B. 3  $\left(\frac{1}{I}\right)$ ; *Proctotrypidae* sp. (A.) D. 2, 3  $\left(\frac{2}{I}\right)$ ; *Pelecinidae* sp. (A.) 3  $\left(\frac{1}{I}\right)$ ; *Braconidae* sp. (A.) E. 3  $\left(\frac{1}{III}\right)$ ; *Myrmica scabrinodes* (A.P.L.) E. 1-3  $\left(\frac{463}{I-IV}; \frac{263}{II}\right)$ .

**Diptera.** *Phronia* (?) *perdita* Mg.\* (P.) E. 1  $\left(\frac{1}{I}\right)$ ; *Dilophus febrilis* L.\* E. 1  $\left(\frac{3}{I}\right)$ ; *Bibio venosus* Mg.\* C. 1  $\left(\frac{13}{III}\right)$ ; *Leptis scolopacea* L.\* B. 1  $\left(\frac{5}{I-III}; \frac{3}{I}\right)$ ; *Tipula ochracea* Mg.\* D. 1  $\left(\frac{1}{I}\right)$ ; *T. lateralis* Mg.\* C. 1  $\left(\frac{3}{I}\right)$ ; *Rhyphus punctatus* F.\* E. 1  $\left(\frac{2}{I}\right)$ ; *Scatophaga stercoraria* L.\* D. 2, 3  $\left(\frac{2}{I}\right)$ ; *Borborus geniculatus* Moq. E. 2  $\left(\frac{1}{I}\right)$ ; *Anthomyia* sp.\* D. 3  $\left(\frac{2}{I}\right)$ ; *Ceratopogen* sp. (L.) A. 1, 3, 4  $\left(\frac{22}{I}\right)$ ; *Camptocladus* sp. (L.) A. 3  $\left(\frac{94}{I-IV}; \frac{40}{I}\right)$ ; *Gnophomyia* sp. (L.) A. 1  $\left(\frac{1}{I}\right)$ ; *Hexatominæ* sp. (L.) 1  $\left(\frac{1}{I}\right)$ ; *Aphiochaeta brevicosalis* Wood\* B. 2  $\left(\frac{37}{I}\right)$ .

## 8. DISCUSSION OF DATA FURNISHED BY FAUNAL CENSUS.

### A. TOTAL FAUNA.

(a) *In Relation to the Different Soil Types.* The total number of invertebrates per sample is much higher than has been indicated by previous workers on soil animal ecology, except in the case of Thompson (1924), who also worked on Aberystwyth soil. This is due, probably, to the further refinement of methods adopted by Thompson and in the present investigation for separating and detecting the soil organisms, especially the Acarina and Collembola.

There is considerable variation in the total number of invertebrates found in the different soil types (Figs. 1 and 2). The Alluvial contained the lowest number and it is suggested that the differences between its numbers and those of the Boulder Clay pasture may be due to the high water content of the former. If this be so it follows that the situation of

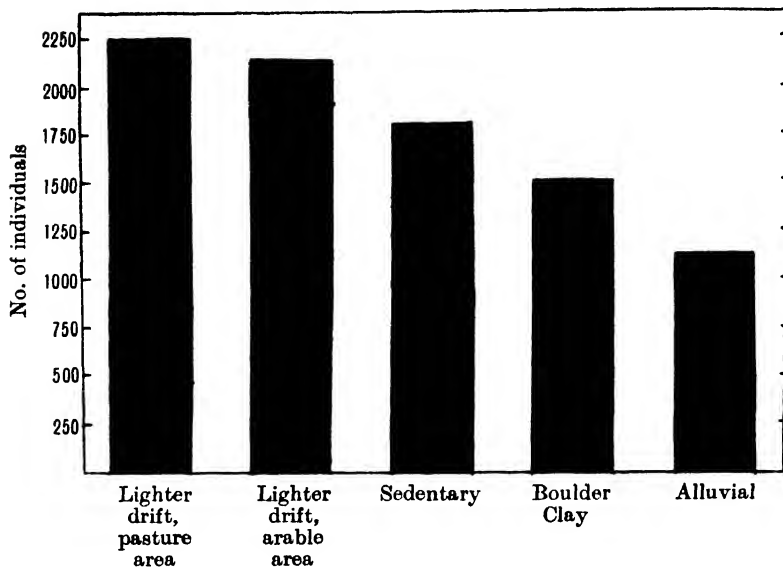


Fig. 1. Relative frequency of occurrence of soil fauna in the five areas.

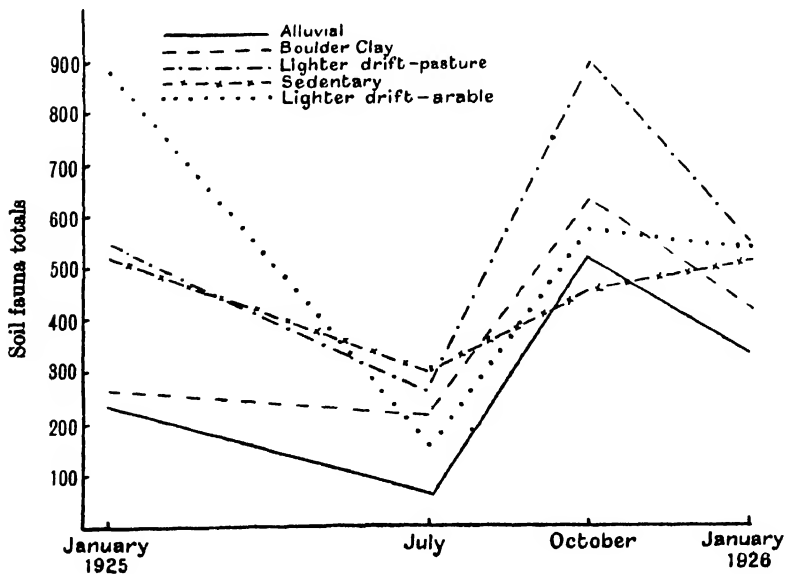


Fig. 2. Total fauna curves.

the land may have a considerable effect on the fauna in that it affects the amount of gravitational water present. Ground water is most destructive to soil inhabitants, not only because the spaces between the soil particles become completely filled with water, thus expelling the free soil air, but the organisms are much more liable to fungoid attacks. It will be noticed that the Lighter Drift soil differs from the Boulder Clay soil mainly in the larger size of soil particles (Tables II and III), and it is therefore probable that the quantitative faunistic difference between the two may be due to a difference in their water-holding power. The latter increases with the decrease in size of the soil particles, and the atmosphere available to subterranean animals differs accordingly in different soils. The degree of aeration of a soil is of equal importance to the fauna indirectly as oxygen is essential for most actions and interactions of the factors operating in the soil.

Ants occurred in three samples examined from the Sedentary area, but have not been included in the totals from which the figures were drawn.

It is seen that there was a marked difference in the total numbers of invertebrates obtained from the Sedentary and Lighter Drift pastures respectively, as illustrated in Fig. 1. This difference was largely due to the lower number found in the sample examined in October from the Sedentary area (Fig. 2). Omitting the October difference, the numbers found were remarkably similar in the two soils. It may be suggested that the difference in October should be associated with the nature of the flora. The predominant grass in the Lighter Drift is *Alopecurus pratensis*, which is a tall and very leafy plant compared with *Cynosurus cristatus*, which is the most abundant grass in the Sedentary area (Table I). The former grass offers the greater shelter and protection, especially in autumn, and is the more efficient in the prevention of undue evaporation of moisture from the soil.

Contrary to the experience of previous workers, cultural operations did not seem to have an appreciable detrimental effect on the total population in the Arable area, as compared with the Lighter Drift pasture. This was due to the higher numbers of Collembola and Myriapoda found in most of the samples from the Arable area. Analysis shows, however, that it is the Collembola, Onychiuridae spp. which are of importance in Lighter Drift arable and Lighter Drift pasture respectively. The relatively high position of the arable curve in January 1925 is due to the high proportion of *Tullbergia quadrispina* present, whereas the high position of the pasture curve in October is influenced by an increase in number of *Onychiurus armatus*.

Fig. 2 shows the quantitative changes in the total fauna for each of the soil types at different times of the year. The great difference in the levels of the curves in July and October might be explained as being related, not only to the normal seasonal variation at these times of the year, but to the unusual dryness of seven weeks previous to the taking of samples in July. This conclusion is further confirmed by the fact that shrivelled dead bodies of Collembola and Symphyla were found in considerable quantities.

The total number found in samples taken in January 1925 and the corresponding time in the following year was almost identical in the case of the Sedentary and Lighter Drift pastures (Fig. 2). In the late spring and early summer 1925, while the investigation was in progress, the drainage of both the Alluvial and the Boulder Clay areas was greatly improved. This led to a remarkable improvement in the conditions of these areas. Appreciable increase is observed in the total fauna of the samples taken in January 1925, as compared with the ones made at the corresponding time in the previous year (Fig. 2). This improvement was not confined to the uppermost layer, but extended down to the third layer.

All the soils under consideration are rich in organic matter, and even the subsoil seems to contain a fair amount, as indicated in Table III (under heading "Loss on ignition"). Though the arable area contains the least amount, yet, excepting the Lighter Drift pasture, this area has the highest amount of organisms. Thus it follows, firstly, that the differences in the amount of organic matter are not the effective factor in determining the total soil population in the pasture areas examined, and secondly, seeing that even the subsoils of the investigated grasslands are nearly as rich in organic matter as the soil of the arable area, it is highly probable that the organic content is not the determining factor in the vertical distribution.

(b) *In Relation to Depth.* The depth at which soil organisms occurred in the five areas investigated is of considerable interest (Fig. 3). That the fauna of the Alluvial area was almost exclusively confined to the uppermost inch layer may probably be associated with the high water level, owing to its low-lying situation. This conclusion is further borne out by the fact that the improved drainage of the ground water into lower levels, made during summer 1925, was followed by a partial restoration of the fauna in the lower levels.

The fauna of the Boulder Clay area, though largely found in the surface inch layer, was more evenly distributed and more abundant in the lower layers than that of the Alluvial soil. Contrary to expectation, the



third layer contained a higher number of individuals than the second layer (Fig. 3). This observation suggested the advisability of making a mechanical analysis, and an estimation of the organic content for each layer separately. The method adopted for the mechanical analysis was the one advocated by Robinson (1922) which is recognised to give a more

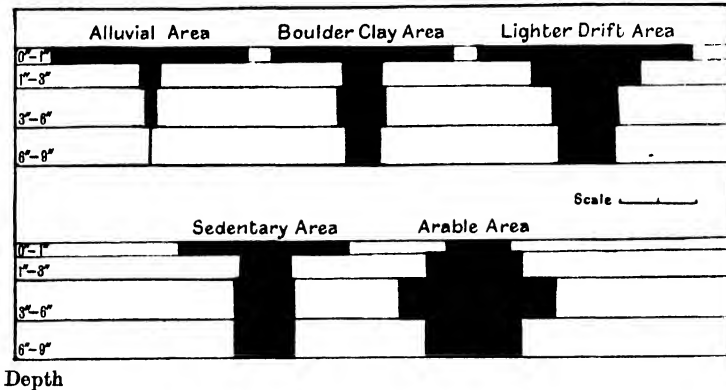


Fig. 3. Distribution in depth of the soil fauna in the five areas.  
Scale, 1 div. = 200 individuals.

exact result than the ordinary agricultural sedimentation methods. A mechanical analysis of representative samples of the layers gave the following results.

Table IV.

*Mechanical Analysis of the Soil of the Boulder Clay area at Different Depths.*

				Depth in inches	
				1-3	3-6
Fine gravel	...	...	3.7	4.0	
Coarse sand	...	...	5.4	4.6	
Fine sand	...	...	10.7	10.4	
Silt	...	...	12.0	13.75	
Fine silt	...	...	38.0	39.5	
Clay	...	...	14.5	13.5	
Moisture	...	...	3.2	3.2	
Loss on ignition	...	...	11.9	10.7	

It will be noted that the mechanical composition and the organic content is apparently insufficient to account for the peculiar vertical distribution of the fauna. However, there is a distinct difference between these layers *in situ*. The upper layer (1-3 in.) presents an appearance of a dense mass of closely packed particles while the lower appears much more

porous. This compactness of the 1-3 in. layer may probably be the result of the interaction of the high clay and fine silt content with the pasture condition. The effect of rain and trampling by the larger grazing animals is, naturally, more marked nearer the surface than at lower levels. The Lighter Drift, being composed of larger sized particles, possesses a much lower degree of plasticity, resulting in higher percentage of the soil population in the 1-3 in. layer, compared with that of the corresponding layer in the Boulder Clay soil.

There is a distinct difference in the distribution in depth of the soil fauna of the Sedentary and Lighter Drift pastures (Fig. 3). This difference may be associated with at least two main factors. One is that the Sedentary soil contains such a high percentage of stones (Table II) that the conditions within the soil can be regarded as being more favourable for the deeper penetration of soil organisms than those of the Lighter Drift pasture. The other factor is that by reason of the predominant shallow-rooted plant species *Cynosurus cristatus* in the Sedentary pasture, the turf or surface covering is less thick, affording freer access of air into the lower layers and more equable conditions at greater depths.

In the arable area there was an increase of soil organisms with increase of depth in the upper six inches of soil, the depth to which arable land is usually cultivated (Fig. 3). This may be related to the loss of moisture, especially in the surface three inches, owing to progressive evaporation. The loose surface soil interrupts the capillary passage of moisture to the surface, and thus tends to a greater conservation in the deeper layers. Also the organic matter in an arable soil is more evenly distributed throughout the soil, and the latter being less compact, owing to the cultural operations, contains a higher percentage of free soil air than a similar soil of a permanent pasture. It therefore seems to be to the advantage of soil organisms in arable land to entrench deeply, so avoiding the consequences of sudden changes of weather conditions.

The tendency of modern investigations has been to show that soil organisms do not descend to greater depths during the winter months. The present data suggest—that in the lighter type of soils the organisms do descend to deeper levels but that in the heavier soil types the reverse is the case. This difference in seasonal behaviour of the fauna of different soil types in regard to vertical distribution has every appearance of being related to differences in the situation and mechanical composition of the soil, both of which in turn are associated with geological formation, and are themselves concerned in regulating the degree of moisture, aeration and temperature.

### B. COMPONENTS OF FAUNA IN RELATION TO THE DIFFERENT SOIL TYPES.

It seems that the laws governing plants in their relation to soils apply in the main to the soil fauna. In the Alluvial area one of the predominant

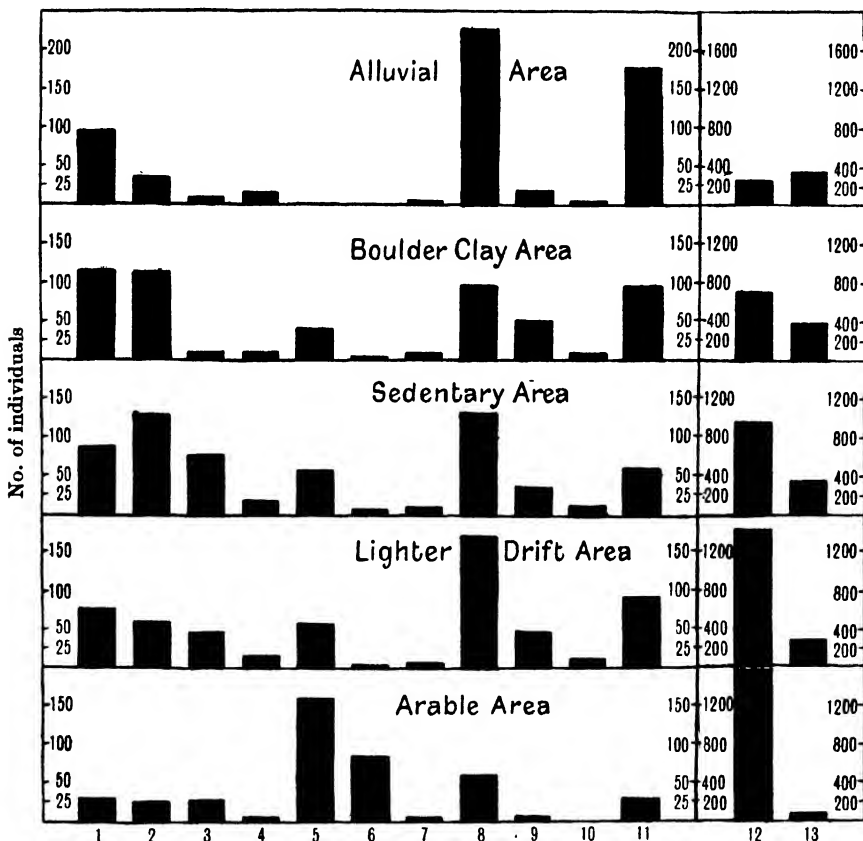


Fig. 4. Relative frequency of occurrence of individual groups.

- 1, Terricola; 2, Terricolae cocoons; 3, Nematoda; 4, Gastropoda; 5, Symphyla; 6, Myriapoda other than Symphyla; 7, Araneida; 8, Acarina; 9, Coleoptera; 10, Rhynchota; 11, Diptera; 12, Collembola; 13, Limicolae.

weeds was *Juncus effusus*, which is essentially a marshland species (Table I), and the majority of the Dipteran larvae also were aquatic or semi-aquatic forms.

It is noticeable (Fig. 4) that in the four pasture areas the Collembola, Oligochaeta (Limicolae) and Acarina were much the most abundantly

represented groups, and that the Oligochaeta (Terricolae), Diptera and Coleoptera were also fairly numerous in the four areas. Gastropoda, Rhynchota and Araneida were poorly represented. Symphyla and Nematoda showed a considerable difference in numbers, the former as well as Chilopoda and Diplopoda not being found in the Alluvial area.

Injurious insects occurred in all the grassland areas. The total numbers of Elaterid larvae recorded were 2, 14, 2, 3, for the Alluvial, Boulder Clay, Sedentary and Lighter Drift pastures respectively. The corresponding figures for Tipulidae larvae were 0, 3, 4 and 6. The larvae of *Athous haemorrhoidalis* were, except for single specimen, confined to the Boulder Clay, while the larvae of *Agriotes obscuris* occurred in all the areas.

It is noticeable that there is a marked difference in the fauna of the arable and pasture areas of the Lighter Drift. Cultivation seems to be associated with considerable increase in the number of Myriapoda and the Collembola, Onychiuridae spp. and with a reduction in the number of other groups, including Collembola other than Onychiuridae spp., represented in Fig. 4. Diplopoda contributed over 75 per cent. of the Myriapoda population (exclusive of Symphyla) in the arable area, and were represented by four species. Of the four species *Brachyiulus (Microbrachyiulus) pusillus* was best represented, forming about 56 per cent. of the total Diplopodan population. It will be noted that the Diplopoda were on the whole most numerous in the surface three inches, while the Chilopoda were more abundant at greater depths, where the soil was richest in fauna suitable for their food.

#### 9. NOTE ON *OLIGOTROPHUS ALOPECURI* AND *APHIOCHAETAE BREVICOSTALIS*.

As many as 235 larvae of a Cecidomyid, *Oligotrophus alopecuri*, occurred in a sample examined from the Alluvial portion of Cae'r Efail. The larvae were of a crimson or pinkish colour and occurred gregariously near the base of *Alopecurus pratensis* grasses between the old sheath and the stem. Members of this species were not found parasitic on any other grasses. Their presence on the plant involved no serious effects except withering of the lower leaf. Before pupating the larvae seem to bore their way through the leaf sheath and pupate on the plant itself. The first adult appeared on March 20th under laboratory conditions, and has been doubtfully classified as *Oligotrophus alopecuri* Reut.

Another interesting case of parasitism was observed during the investigation. A number of specimens of the Phorid, *Aphiochaetae brevicostalis* Wood, were reared from a *Tipula* sp. larva. The latter when

obtained were alive. This parasitic Dipteran has only been recorded and bred before from dead snails; it may prove to be of economic significance.

#### 10. SUMMARY.

1. Samples of soil were taken from four distinct soil types of a permanent pasture, and one of an arable land at the College Farm, Nantcellan Fawr, Aberystwyth. It is believed that the areas chosen are representative of their type in the district.

2. The pasture areas studied have been in pasture for at least forty years, while the arable area has been continuously cultivated, apart from an occasional rest in grass of from two to three years, for an equally long period.

3. Mechanical, chemical, botanical and faunal analyses were made.

4. The method of investigating the fauna consisted in taking samples of soil of standard size. The soil was removed in layers so that the approximate depth at which the insects and other invertebrates occurred, could be stated.

The soil of each layer, after a preliminary examination in the laboratory, was sieved in water. In order to deal as effectively as possible with the smaller organisms the residue left on each sieve was transferred to a shallow dish, and covered with water when the remaining smaller animals either floated on the surface of the water, or remained on the surface of the residue at the bottom of the dish.

5. The greatest number in the pasture areas, both of insects and other invertebrates, occurred in the surface inch layer, but some species were found in larger numbers at a greater depth. Thus the greatest number of Symphyla were found at a depth of six to nine inches and of *Tullbergia quadrispina* usually at a depth of three to six inches.

6. Some species, such as *Myrmica scabrinodes* were confined to the Sedentary area, and *Folsomia quadrioculata* to the Alluvial area, while other species occurred in all the areas. Collembola, *Onychiurus armatus*, while occurring in all the areas, were more common in the Lighter Drift areas.

7. The examination of cultivated land revealed a considerable increase in Symphyla, Diplopoda and some Collembola (*Tullbergia* sp.) and a reduction in the number of other Collembola, Oligochaeta, Acarina, Coleoptera and Diptera, compared with a pasture area of a similar soil type.

8. While qualitative difference may exist between the fauna of the several areas, the main differences concern rather the proportions in

## 322 *Invertebrate Fauna of Pasture and Arable Land*

which the various organisms occurred. There is decidedly less difference both qualitatively and quantitatively between the animal associations than there is between the plant associations.

9. An attempt was made to correlate the faunal data obtained in each area with the general environmental conditions. It was suggested that the differences in horizontal and vertical distribution were probably associated:

(i) Mainly with situation and mechanical composition of the soil, which in turn determines the degree of moisture, aeration and temperature at any given region within the soil.

(ii) With the nature of the flora, in that it affects the density and thickness of the surface turf and means of shelter and protection above ground as well as influencing the degree of evaporation.

(iii) With the depth at which particular food occurred especially to carnivorous animals, such as Chilopoda.

10. Injurious insects occurred in the four pasture areas. The larvae of Elateridae and Curculionidae were found in all but were scarce in the Alluvial. The larvae of Tipulidae were absent from the Alluvial, but were present in the other three.

11. Parasitism was infrequent, but a larvae of *Tipula* sp. was found attacked by *Aphiochaeta brevicostalis* Wood and *Alopecurus pratensis* by *Oligotrophus alopecuri* Reut. (?).

### REFERENCES.

- BRAUER, A. (1909). *Die Süßwasserfauna Deutschlands*. Heft. Mollusca.  
BUCKLE, P. (1921). A Preliminary Survey of the Soil Fauna of Agricultural Land. *Ann. App. Biol.* viii, 3 and 4.  
CAMERON, A. E. (1913). General Survey of the Insect Fauna of the Soil. *Journ. Econ. Biol.* viii, pt. 3.  
— (1916). The Insect Association of a Local Environmental Complex in the district of Holmes Chapel, Cheshire. *Trans. Roy. Soc. Edin.* lii, pt. 1 (No. 2).  
EDWARDS, J. (1896). *The Hemiptera-Homoptera of the British Islands*.  
FOWLER, W. W. (1887). *British Coleoptera*, i-vi.  
JEFFREYS, J. G. (1914). *British Conchology*, i.  
LUBBOCK, Sir A. (1871). Monograph on Collembola and Thysanura.  
MALLOCH, J. R. (1917). Preliminary Classification of Diptera exclusive of Puparia, based upon Larval and Pupal Characters. *Bull. Illin. Stat. Lab. Nat. Hist.* xii, pt. 3.  
MORRIS, H. M. (1920). Observations on the Insect Fauna of Permanent Pasture in Cheshire. *Ann. App. Biol.* vii, 2 and 3.  
— (1922). The Insect and other Invertebrate Fauna of Arable Land at Rothamsted. *Ibid.* ix, 3 and 4.  
— (1921). Larval and Pupal Stages of the Bibionidae. *Bull. Ent. Res.* xii, 3.

- RIMMER, R. (1907). *Land and Freshwater Shells of the British Isles*. (Edinburgh.)
- ROBERTS, A. W. RYMER (1919). On the Life History of "Wireworms" of the Genus *Agriotes*, Esch., with some notes on that of *Athous haemorrhoidalis* F. *Ann. App. Biol.* VI, 1 and 3.
- (1922). *Ibid.* IX, 3 and 4.
- ROBINSON, G. W. (1922). A New Method for the Mechanical Analysis of Soils and other Dispersions. *Journ. Agric. Sci.* XII, 306-321.
- SAUNDERS, E. (1896). *The Hymenoptera-Aculeata of the British Islands*. (London.)
- THOMPSON, M. (1924). The Soil Population. An Investigation of the Biology of the Soil in certain districts of Aberystwyth. *Ann. App. Biol.* XI, 3 and 4.
- WINGATE, W. J. (1906). A Preliminary List of Durham Diptera. *Trans. Nat. Hist. Soc. Northumb. Durham and Newcastle*, II.

(Received September 5th, 1928.)

# INVESTIGATION ON *HETERODERA SCHACHTII* IN LANCASHIRE AND CHESHIRE

PART I. THE INFESTATION IN CERTAIN AREAS AS REVEALED BY CYST COUNTS; AN ESTIMATION OF THE ERRORS INVOLVED IN THE TECHNIQUE AND A CORRELATION WITH INTENSITY OF DISEASE

By A. M. SMITH, B.Sc., Ph.D., A.I.C.

(*Adviser in Agricultural Chemistry, Manchester University.*)

AND

E. G. PRENTICE, B.Sc.

(*Zoology Department, Manchester University.*)

(With 4 Text-figures.)

## INTRODUCTION.

ALTHOUGH Kühn<sup>(3)</sup> as early as 1881 recognised that *Heterodera schachtii*, the beet eelworm, could occur on the roots of potato plants, it was not until 1913 that Zimmermann definitely recognised it as a serious parasite of the potato and, in 1920, published a paper<sup>(6)</sup> dealing with its occurrence and the damage which it produced on the potato crop in his own country. A few years later, Morgan<sup>(4)</sup> recorded the presence of a similar disease in Lincolnshire, but it has been recognised at many centres all over Britain.

As yet no satisfactory account of the life history of *Heterodera schachtii*, as it occurs on potatoes, has been published. Most investigators have been content to assume that the course of the life history of this strain on potatoes is identical with or very similar to that of the much better known strain on sugar beet, a very full description of the structure and development of which is given by Strubell<sup>(5)</sup>.

## OBJECT OF INVESTIGATION.

The failure of many potato crops in recent years has been attributed to "eelworm disease," but much doubt has been expressed as to the actual rôle of the eelworm in the disease. If there is a relationship between the eelworm infestation of the soil and plant disease, it is necessary to have a



satisfactory technique for measuring the infestation. The most obvious and direct method appears to be the counting of the encysted females, preferably in samples of soil collected during the winter months. The work reported in this paper was carried out in order to arrive at a suitable method of determining infestation by sampling and counting, with a computation of the errors involved. An attempt was then made to correlate differences in cyst counts with variations in intensity of disease in the previous potato crop.

#### AREA INVESTIGATED.

Although, as subsequent pages will show, the occurrence of "eelworm" seems to be fairly widespread in Lancashire and Cheshire, attention was directed in the first instance to a few localities where potatoes are probably the most important crop in a short rotation and where the rotation could be lengthened only with difficulty. In the Mersey basin to the west of Manchester lies a fairly extensive area of peat. A considerable portion of this has been reclaimed by draining, burning the surface vegetation and cultivating. The resulting peat soil, frequently modified to a great extent by very heavy applications of marl and city refuse, is excellent for market garden produce and potatoes, and is farmed intensively rather than extensively. Usually potatoes are followed by oats and seeds, occasionally wheat comes into the rotation, but on the other hand potatoes frequently appear every second year, cabbage, celery, and so on, being taken up irregularly. Late frosts are sometimes troublesome and may damage the crops to the end of June so that late varieties, planted about the beginning of May, are always grown. A potato disease has been known to exist in this area since 1922 but was usually confined to comparatively small patches in the fields. In 1927 cysts of *Heterodera schachtii* were recognised as being associated with diseased plants and there was evidence that the trouble was reaching important dimensions, as fairly large tracts of ground yielded but a fraction of the normal crop.

In West Lancashire a second area of peat occurs, and, bordering on the peat proper, there are to be found soils which are perhaps best described as peat sands. Apparently, a thin layer of peat, together with heavy dressings of organic manures, has become incorporated with the first 15 inches or so of underlying sand (usually white) to give a dark grey soil with a sandy or sandy loam texture. On account of the sand substratum, which is usually at least three feet thick, these soils are well drained. In this area the farming is rather more extensive and varied than in the former, but potato growing is a most important source of

## 326 *Heterodera schachtii* in Lancashire and Cheshire

revenue to the farmer. In this area *Heterodera schachtii* was identified in 1923, and during the last two years "eelworm" disease has been observed in several localities.

### TECHNIQUE.

*Field.* Generally speaking, a strip about 5 yards wide, and running through the centre of a patch previously known to be affected with disease, was selected for examination. The strip was subdivided into lengths varying from 10 to 15 yards so that each plot sampled had an area of from 50 to 75 sq. yards. At least ten borings to plough depth were made at intervals over each plot, a soil auger being employed for the purpose. Except in two cases which will be discussed later, the borings were mixed to give a composite sample. This method was devised to get a representative sample of soil to plough depth and overcome the difficulty due to the fact that some centres had been ploughed whilst others remained unploughed at the time of sampling.

*Laboratory.* The composite samples were spread out in the laboratory and allowed to reach an air-dry condition. They were then broken up and the material passing through a 2 mm. sieve was employed in the examinations. For cyst counts, ten samples of 10 c.c. were taken, by the usual method of quartering, from each composite sample. The cysts were removed from the soil by the method described by Morgan (4). The sample of soil was placed in a Stohmann shaking bottle or a standard flask, and shaken with about 200 c.c. of water for 4 or 5 minutes. The flask was then filled up with water and allowed to stand until the cysts had floated with undecayed vegetable matter to the surface. The floating material was then thrown on to a filter paper and the cysts counted under a low power lens. A number of cysts adhere to stones and fragments greater than 2 mm. and are therefore lost in the above method. To estimate the loss incurred, the material greater than 2 mm. in diameter was examined in ten cases for peat soils, and in four cases for sandy soils. The greatest loss was 3.3 per cent. for a peat soil and 1.2 per cent. for a sandy soil, the respective averages being 2.5 per cent. and 0.9 per cent. As will be seen from the tables which follow, these figures, which were fairly constant for the two soil types, are scarcely worthy of serious consideration.

### SOURCES OF ERROR.

The counts made as described are liable to two important sources of error namely, (a) the field error due to taking ten borings to form a composite sample representative of a plot, and (b) the laboratory error due to

the sampling and counting of the sieved air dried soil. An attempt has been made to express those errors in the form of percentage standard error.

*Laboratory error.* Sufficient data are available to form a good estimate of the standard error due to laboratory sampling and counting, for over 90 samples, yielding about 900 counts, were carefully examined. Table I is typical of a series of samples and is set out at length to show the variation found in the individual counts of a sample and the method of arriving at the standard error of each mean.

Table I.

*The cyst counts in a series of ten composite samples and the estimation of the laboratory standard error.*

Sample	120	A	B	C	D	E	F	G	H	I	J
	32	44	41	57	47	41	48	40	54	35	
	34	51	42	48	42	36	50	52	28	25	
	44	43	30	49	53	48	54	61	52	28	
	31	44	29	42	50	36	48	49	41	37	
	35	39	39	42	47	44	59	65	48	42	
	31	39	39	49	57	44	68	59	40	30	
	21	44	33	37	58	42	50	64	58	30	
	40	42	35	52	45	30	51	55	42	29	
	33	50	37	45	40	43	57	48	64	30	
	32	49	50	52	52	34	46	40	43	27	
$\bar{X}$	33.3	44.5	37.5	47.3	49.1	39.8	53.1	53.3	47.0	31.3	
$S(X - \bar{X})^2$	328.0	162.0	348.0	312.0	325.0	181.0	399.0	748.0	874.0	240.0	
$\sigma^2$	36.4	18.0	38.7	34.7	36.1	20.1	44.3	83.1	97.1	26.7	
$\sigma^2/\sqrt{10}$	1.91	1.34	1.97	1.86	1.90	1.42	2.10	2.88	3.12	1.63	
% error	5.73	3.02	5.25	3.94	3.87	3.56	3.96	5.41	6.63	5.22	
$\chi^2$	9.85	3.64	9.28	6.60	6.62	4.55	7.52	14.04	18.60	7.67	

*Average percentage error = 4.66.*

$\bar{X}$  = the arithmetic mean of each set of ten individual counts.

If  $X$  = any one count,  $(X - \bar{X})$  is the deviation from the mean.

$S(X - \bar{X})^2$  = sum of the squares of the deviations.

$\sigma^2$  = the variance =  $\frac{S(X - \bar{X})^2}{n - 1}$  for small samples, when  $n$  equals the number of counts (2).

$\sigma$  = the standard deviation and  $\sigma/\sqrt{10}$  = the standard error of the mean.

$$\chi^2 = \frac{S(X - \bar{X})^2}{\bar{X}}$$

The results of every sample have been subjected to the same treatment to get an estimate of the standard error due to sampling and counting in the laboratory. For each series of plots, the percentage error has

## 328 *Heterodera schachtii* in *Lancashire* and *Cheshire*

been averaged<sup>1</sup> for those counts of more than ten cysts per 10 c.c. of soil. That limit has been chosen arbitrarily in order to exclude those samples taken from a strip running beyond an area actually infested and those samples in which the number of cysts was so small as to make the standard error quite abnormal. Table II gives a summary of the results.

Table II.

*The percentage standard errors due to laboratory technique  
and the indices of dispersion.*

Soil type	Series	Number of samples	Average standard error per cent.	<i>Sn</i>	$\chi^2$
Peaty sand	23-32	10	7.34	90	93.11
	124 A-124 E	4	6.82	36	29.21
	66-75	3	6.44	27	30.92
	Weighted mean		7.06		
Peat	46-55	4	6.22	36	34.01
	113 A-113 C	3	7.89	27	42.44
	11-20	10	4.31	90	61.44
	111 A-111 D	4	9.02	36	40.82
	112 A-112 D	3	8.49	27	39.67
	6-10	2	5.49	18	18.83
	56-65	10	5.86	90	84.01
	120 A-120 J	10	4.66	90	88.37
	101-110	10	5.30	90	91.61
	Weighted mean		5.76		
Total		73	—	657	654.44

The figures in Table II show that when sandy soils are considered the standard error due to laboratory technique is of the order of 7.1 per cent., whilst for the peat soils, which incidentally were found to have much greater counts, the error is in the neighbourhood of 5.8 per cent. That laboratory error appears to be large, but a more careful consideration of the position indicates that it is what might be expected. If a particle is taken from the soil, the chance of its being a cyst is very small indeed: if, therefore, samples consisting of large numbers of particles are taken at random from a bulk sample, the numbers of cysts in those samples should be distributed according to a Poisson series. An agreement of the results with the theoretical distribution affords a test of the suitability of the technique, and they have been submitted to an analysis similar to that employed by Fisher, Thornton and Mackenzie(1). For all Poisson series

<sup>1</sup> Since only an estimate of the error was desired the arithmetic mean has been taken.

the variance is numerically equal to the mean, and the index of dispersion,  $\chi^2 = \frac{S(X - \bar{X})^2}{\bar{X}}$ , is distributed in a known manner so that, for every value it assumes, there is a corresponding value  $P$  representing the probability that  $\chi^2$  will be exceeded by chance. In other words, it is possible to test the agreement between the results obtained and those expected. As a first approximation  $\sigma^2$  was plotted against  $\bar{X}$  and Fig. 1 shows that the majority of the points lie fairly closely to the line representing a true Poisson series. With a single sample of ten counts the range of variation of  $\chi^2$  is too great to be of much value, but the sum of any number of quantities  $\chi^2$  is distributed in the  $\chi^2$  distribution, and it is therefore at least possible to test if the variability from expectation is normal. The values of  $\chi^2$ , calculated as in Table I for each sample of ten counts, have been summed for each series of samples, Table II. In this case  $Sn$  is equal to the sum of the various values of  $n$  for the separate samples,  $n$  being one less than the number of counts.

To test if the value 654.44 for  $\chi^2$  is normal for  $n = 657$ , use has been made of the fact that for such a large value for  $n$ ,  $\sqrt{2\chi^2}$  is approximately normally distributed about  $\sqrt{2n} - 1$  with unit standard deviation. In this case

$$\begin{aligned}\sqrt{2\chi^2} &= 36.18, \\ \sqrt{2n} - 1 &= 36.24, \\ \text{Difference} &= -0.06.\end{aligned}$$

The difference is much less than the standard deviation, so that the variability between parallel counts is quite normal.

Finally, values for  $\chi^2$  are set out in Table III at intervals alongside the expected values taken from a  $\chi^2$  table(2).

Table III.

*Comparison of observed and expected distribution of  $\chi^2$ .*

$\chi^2$	Expected	73 % expected $m$	Observed $m + x$	$x^2/m$
4.168	10	7.3	7	0.012
5.380	10	7.3	7	0.012
6.393	10	7.3	6	0.232
8.343	20	14.6	22	3.750
10.656	20	14.6	18	0.792
12.242	10	7.3	2	3.849
14.684	10	7.3	5	0.725
and over	10	7.3	6	0.232
Total	—	73.0	73	$\chi^2 = 9.604$ $P = 0.2$

### 330 *Heterodera schachtii* in Lancashire and Cheshire

The agreement is very good. By taking eight groups the probability of obtaining a worse fit by chance from normal data is about .2, so that there is no significant deviation of the values from expectation. The analyses of the results seem to indicate, therefore, that most of the sets meet the conditions required by samples from the Poisson series and that, therefore, the technique was satisfactory and the mean value for each set of counts a reliable estimate of the number of cysts of *Heterodera schachtii* present in the soil sample. It follows that if the technique were perfect there would still be an inherent percentage standard error equal to  $\sqrt{\frac{20.44}{10}} \times \frac{100}{20.44} = 6.99$  for the peaty sands and  $\sqrt{\frac{32.18}{10}} \times \frac{100}{32.18} = 5.57$  for the peats, where 20.44 and 32.18 are the respective means when samples having counts less than 10 are omitted as previously. The values found compare very favourably with those calculated and may therefore be employed with some confidence as giving a measure of the laboratory error.

*Field error.* A large variation in the numbers of cysts from point to point on a plot is to be expected. To get figures for that variation in order to arrive at an estimate of the error involved in making a composite sample of ten borings, the following experiment was carried out. Ten samples were taken from each of the plots 124 C and 11, and examined separately. Ten counts were made for each sample. Table IV summarises the results.

The values 13.1 and 10.6 represent the percentage standard errors of the mean of a total of  $mn$  counts on  $n$  borings

$$= \sqrt{\frac{\sigma_L^2}{mn} + \frac{\sigma_F^2}{n}} \quad \dots\dots(A),$$

where  $m = n = 10$ ,

$\sigma_L/\sqrt{m}$  = the percentage standard deviation of the mean of  $m$  counts.

$\sigma_F/\sqrt{n}$  = the percentage standard deviation of the mean of  $n$  borings.

The value for  $\sigma_F/\sqrt{n}$ , representing the field error, has been calculated from equation (A).

*Total error.* The "total standard error" of the mean of  $m$  counts on a composite sample of  $n$  borings is then equal to  $\sqrt{\frac{\sigma_L^2}{m} + \frac{\sigma_F^2}{n}} = 14.7$  per cent. for the peaty sand and 11.7 per cent. for the peat.

The extent of those errors is due largely to the abnormal variations of samples 32 and 106 from the respective means. If those samples are

Table IV.

*Variation in cyst content over two small plots and estimation of field errors.*

Peaty sand plot 124 C			Peat plot 11*		
Sample	Mean of 10 counts ( $\bar{X}$ )	( $X - \bar{X}$ ) <sup>2</sup>	Sample	Mean of 10 counts ( $\bar{X}$ )	( $X - \bar{X}$ ) <sup>2</sup>
23	16.3	7.3	101	25.8	100.0
24	14.0	25.0	102	32.0	4.8
25	11.4	57.8	103	25.5	106.1
26	10.6	70.6	104	45.7	98.0
27	15.9	9.6	105	35.1	0.5
28	22.3	10.9	106	62.7	723.6
29	17.8	1.4	107	26.3	90.3
30	19.5	0.3	108	45.3	94.1
31	24.3	28.1	109	31.8	16.0
32	37.5	342.0	110	27.9	62.4
Total ...	189.6	553.0	—	358.1	1295.8
Mean $\bar{X}$ ...	18.96	—	—	35.8	—
Variance ( $\sigma^2$ ) ...	...	61.4			143.9
Standard error of mean ( $\sigma/\sqrt{10}$ )		2.48			3.79
Percentage standard error ...		13.1			10.6
Percentage $\sigma_L/\sqrt{10}$ ...		7.3			5.3
Percentage $\sigma_F/\sqrt{10}$ ...		12.8			10.5
Percentage "total error" ...		14.7			11.7

\* Samples 101–110 were taken 6 months later than composite sample 11.

excluded the "total standard errors" become respectively 11.1 per cent. and 9.1 per cent. The differences are considerable and serve to show how the large variation in infestation over a small plot may influence the results. It would be advisable to increase the number of borings taken to make the composite sample. For example, if the sample were obtained from 20 borings and 10 counts were made, the error of the mean would be reduced to about 9 per cent. for the peats. It is doubtful if increasing the number of borings beyond 20 would serve much useful purpose since a large increase in the size of the sample to be handled in the laboratory would make satisfactory manipulation difficult. As evidence of the fluctuations actually obtained, the following results are of some interest. Plot 124 C was sampled in the usual way, the mean of the ten counts being 19.7 compared with 18.96 for the ten samples 23–32, or 16.9 for the nine samples 23–31. Plot 46 overlapped plot 113 B and the respective means were 23.5 and 25.0. In both cases, therefore, the difference between duplicates was quite insignificant, being less than the standard error.

Taking the standard error as about 14 per cent. for the peaty sands

## 332 *Heterodera schachtii* in Lancashire and Cheshire

and 11 per cent. for the peats, it is now possible to review the results in greater detail.

### DISCUSSION OF RESULTS.

In order to simplify comparison and avoid unnecessary repetition all the results have been summarised and are presented in Table V. Each cyst count represents the arithmetic mean of ten counts; the samples comprise the following series. Series 23-32, to which reference has already been made, consists of ten samples from plot 124 C. The series 124 A-E lies in a strip through a small field which has been devoted for 8 years, with one break, to continuous cropping with potatoes. The soil is a peaty sand in type, containing about 10 per cent. of organic matter, and a heavy dressing of farmyard manure is given annually. Cysts of *Heterodera schachtii* were found on the roots of the potato plants in 1923 but there was no apparent disease of the shoots. Last season there was still some doubt as to whether there was any disease showing in the aerial parts of the plants, except in one corner of the plot 124 C. Taking the average standard deviation of one plot as 2.2 (14 per cent. of 15.8, the mean of the five plots) and the standard error between two

Table V.

*Figures for cyst counts of soils examined.*

No.	c.c.	No.	c.c.	No.	c.c.	No.	c.c.	No.	c.c.
23	16.3	124 A	17.0	11	46.7	111 A	12.5	6	29.7
24	14.0	124 B	14.8	12	41.2	111 B	10.6	—	—
25	11.4	124 C	19.7	13	30.5	111 C	20.1	10	42.0
26	10.6	124 D	18.6	14	35.0	111 D	13.9	—	—
27	15.9	124 E	9.0	15	32.4	—	—	56	45.7
28	22.3	—	—	16	32.3	112 A	21.7	57	33.8
29	17.8	46	23.5	17	27.9	112 B	19.7	58	21.4
30	19.5	47	26.1	18	30.1	112 C	16.0	59	23.9
31	24.3	48	24.9	19	44.9	112 D	6.6	60	22.5
32	37.5	49	7.4	20	40.2	—	—	61	23.3
—	—	50	19.0	—	—	120 A	33.3	62	25.5
66	8.4	51	7.6	101	25.8	120 B	44.5	63	24.6
67	3.8	52	8.1	102	32.0	120 C	37.5	64	20.0
68	27.9	53	1.9	103	25.5	120 D	47.3	65	18.8
69	40.8	54	0.3	104	45.7	120 E	49.1	—	—
70	19.0	55	0.2	105	35.1	120 F	39.8	—	—
71	4.3	—	—	106	62.7	120 G	53.1	—	—
72	0.6	113 A	21.0	107	26.3	120 H	53.3	—	—
73	0.1	113 B	25.0	108	45.3	120 I	47.0	—	—
74	0.1	113 C	29.2	109	31.8	120 J	31.3	—	—
75	0.0	—	—	110	27.9	—	—	—	—

c.c. represents number of cysts per 10 c.c. soil.



plots as  $\sqrt{2} \times 2.2 = 3.1$ , the only plot which may be regarded as differing significantly from any of the others is 124 *E*; the difference between 9 and the next but one highest count is 2.56 times the standard error of the difference between two plots. In other words,  $P = .01$  and the odds

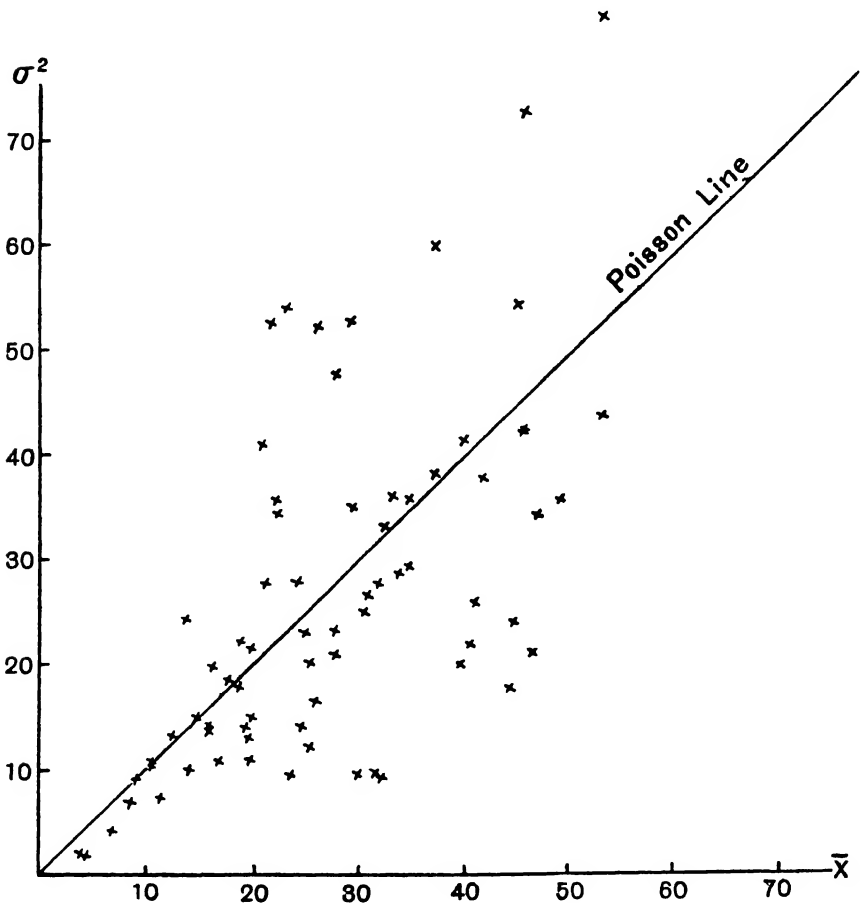


Fig. 1. Diagram representing association between  $\bar{X}$  and  $\sigma^2$  for cyst counts.

against that difference being exceeded as a fluctuation of sampling is about 99 to 1. The infestation of the other four plots may be said to be uniform, whilst the difference between 124 *B* and 124 *E* is barely significant.

Series 66-75 refers to a strip, 100 yards in length, through a field near Ormskirk, the soil being similar to that described above. In 1926

the potato crop was normal except for an isolated patch where the shoots were so diseased that they did not attain a height of 6 inches while the tubers were so small that they were not harvested. The disease had not previously been noted in that field. The usual crop rotation practised in the district is potatoes, wheat, oats, seeds, but this field was again planted with potatoes in 1927. The manuring was considerably altered and consisted of 2 cwt. each of ammonium sulphate, steamed boneflour and superphosphate per acre instead of the usual dressing of 20 tons of city refuse with 1 cwt. each of ammonium sulphate and steamed boneflour. The affected patch observed in 1926 bore, in 1927, a yield of tubers estimated at 60 per cent. normal and the diseased condition of the foliage was not very pronounced. The area of the patch had not increased. Plot 69 of the strip coincided roughly with that patch. There is no need to analyse the results further for there exists a significant increase in number of cysts to plot 69 and then a gradual decrease to nothing, the maximum infestation occurring on the affected patch (Fig. 2).

Series 46-55 consists of a strip, also 100 yards in length, running through a field on Barton Moss, the soil being decomposed peat modified by marling and city refuse, and overlying raw humus. On ploughing this type of soil for potatoes, it is customary to turn up one or two inches of the raw humus which gradually decomposes. The level of the moss land, partly for this reason and partly on account of continued drainage, has fallen considerably since cultivation was started. A 3-course rotation is practised on this farm, the potato crop being followed by oats and then seeds. The manuring for potatoes is usually about 30 tons of city refuse and 2 cwt. of phosphate. Lime is generally applied prior to cultivation for potatoes. There is no history of disease in this particular field prior to 1927 when a patch of dimensions 5 by 30 yards (113 *B*) occurred near one end. On this patch the amount of foliage of the potato plants was only about one-fifth of that in the immediate neighbourhood. As far as the aerial parts of the plants and yield of tubers were concerned, there was no apparent disease outside the patch, although cysts were found in large numbers on the roots of plants many yards distant. The accompanying sketch, Fig. 3, shows the position of the affected area and the two series 46-55 and 113 *A-C*. There is no significant difference between plots 113 *A*, 113 *B*, and 46-48; those, however, have figures significantly greater than all the other plots except 113 *C*. Plots 49, 51, 52 may be regarded as uniform but significantly less infected than plot 50, and significantly more infected than 53, 54, 55. There is, therefore, an undoubted general diminution of infestation away from the diseased area.

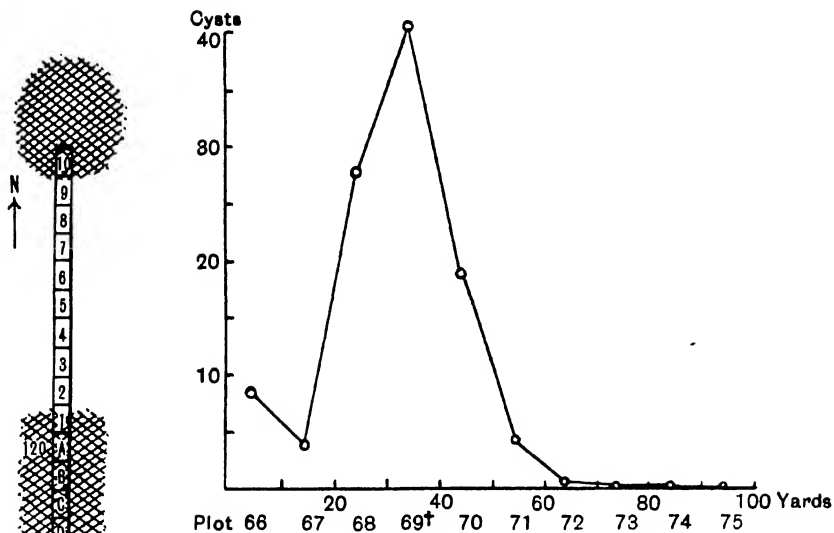


Fig. 2. Infestation through series 66-75: † affected area.

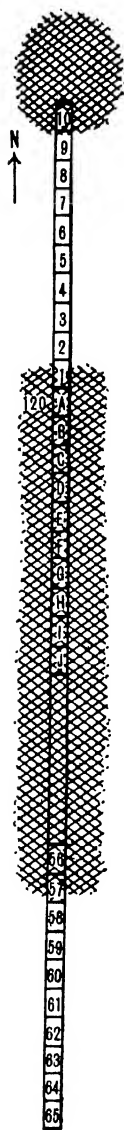


Fig. 4. Location of plots: shaded areas showed reduced yields.

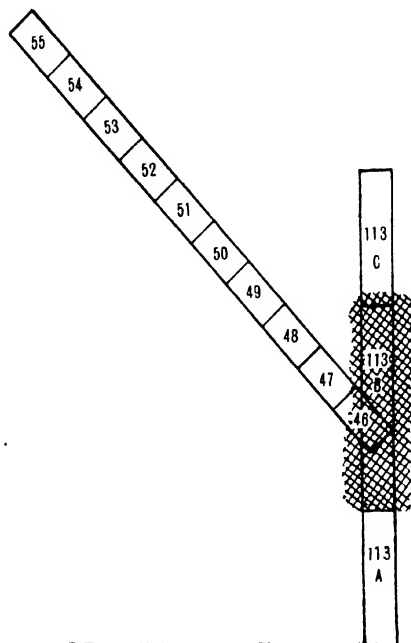


Fig. 3. Position of plots 113A-C, 46-55: much affected area shaded.

### 336 *Heterodera schachtii* in Lancashire and Cheshire

The series 11-20 and 111 *A-D* are from a field on which the soil, rotation and manuring conditions are essentially the same as those in the last case considered except that lime is generally applied prior to sowing out with seeds. A diseased condition of the potato crop was observed in 1924; but it was not until 1927 that cysts of *H. schachtii* were recognised as being associated with the diseased plants. Three centres of disease were observed in 1927. Two of these areas were fairly extensive but the third was small. The disease on the third, however, was so intense that the crop was practically a failure and was harvested early. The series of samples 11-20 were taken from a strip of plots each 5 yards wide and 10 yards long, running through that area. From 11 to 15 the disease was bad, the worst place being in the locality of plot 11. Plots 16, 17, 18 were over an area on which there was no apparent disease. The records are indefinite regarding plots 19 and 20. Plots 111 *A-D* extend through one of the areas less affected. Nothing of a definite nature can be said regarding those series except that the cyst infestation is significantly greater in the series 11-20. In the series 11-20 the figure for plot 11 is greater than those for the six plots 13-18 by amounts which are quite significant. On the other hand, those six plots are essentially uniform in cyst content. In this series, therefore, there appears to be, as far as the information goes, some association between intensity of disease and cyst content. In the other case, 111 *A-D*, the disease was not very bad and was evenly distributed.

The series 112 *A-D* runs across a small field on Barton Moss, the soil conditions being similar to those in the last two cases. The farm is devoted mainly to market garden produce but potatoes are grown usually every third year. Heavy dressings of city refuse are applied and liming is carried out frequently. In 1926 disease was present to a slight extent on plot *A* but was absent from the others. In 1927 half of plot *A* was devoted to cabbages but the rest of the strip had potatoes. Disease was bad over plot *B* but gradually diminished through *C* and was very slight over *D*. The figures for plots *A* and *B* are not significantly different and the difference between plots *A* and *C* is just significant, being about twice the standard deviation between two plots. Plot *D* is certainly less infected. It would again appear, therefore, that there is an association between intensity of disease and cyst infestation.

The samples for the series 1-10, 56-65, and 120 *A-J* were taken from a strip running through a field on Carrington Moss. The soil, rotation and manuring are similar to those already mentioned for peat soils with the exception that lime is not applied. Disease had occurred in patches in

1922; in 1925, the next year for potatoes, the disease was very marked, several of the patches observed in 1922 having run together to form large oval-shaped areas, yielding less than four tons per acre of tubers (40 per cent. normal). The accompanying sketch, Fig. 4, indicates the principal features of those series. The disease was not quite uniform through the plots, and while small patches, where the crop was an entire failure, occurred irregularly, the disease gradually diminished north towards 120 A and the crop was normal about plots 5 and 6. Taking 56-65 into consideration first there is certainly a sudden drop in cyst count from plot 56 to plot 57 and from that to plot 58; the differences between 56 and 57, 57 and 58, are three times the standard error between two plots and are therefore significant. The plots 58-65 on the other hand may be regarded as uniform, the greatest difference being 6.7, which is less than twice the standard error. It is of interest to observe that an examination of 230 cysts from the above series showed that 31 per cent. were empty and black in colour. They may have been the product of the 1922 crop.

The infestation on plot 10 is significantly greater than that on plot 6; the results for the other plots of this series are not available. The series 120 A-J is most irregular, a fact which seems to fall into line with the observations on the disease. Since these observations were not very detailed, however, no purpose would be served by closer examinations of the cyst counts. It is noteworthy, however, that as in other cases, although cysts were invariably present in areas suffering from disease, they were also to be found in the vicinity of plants which seemed to be quite healthy.

#### *Miscellaneous Samples.*

From a stock of soil samples taken in the course of general advisory work, fourteen, from land used for potato growing, were selected at random. Cysts of *Heterodera schachtii* were found in seven of those samples and the counts are submitted (Table VI) merely to show that

Table VI.

*Cyst counts of soils from different areas.*

No.	Soil type	Locality	Cysts per 10 c.c.	pH
S 16	Sandy loam	Knutsford	3.6	5.56
S 47	Clay peat	Worsley	0.7	4.90
84	Peat	Halsall	2.7	4.94
95	Peaty sand	Bickerstaffe	9.1	4.76
96	Peaty sand	Bickerstaffe	0.4	6.22
128	Peaty sand	Bickerstaffe	0.8	6.83
130	Heavy loam	St Helens	3.5	7.88

## 338 *Heterodera schachtii* in Lancashire and Cheshire

the nematode is not confined to certain limited areas but appears to exist throughout the province.

### CONCLUSIONS.

A study of the results as a whole indicates that there is a positive association of intensity of disease of the plants and cyst content of the soil in those cases where the disease has been observed recently. The figures for the two series 66-75 and 46-55 on different soil types are fairly conclusive in that respect. Where, however, the disease was noted several years ago that association is no longer always evident, for a moderately high cyst content may exist where there is no obvious disease. There would, therefore, appear to be some other factor, or factors, at work. A high susceptibility of the young plants to the effects of eelworm attack and a tendency for the majority of the larvae which hatch out later in the season to be attracted to the healthy and necessarily more mature plants, might be advanced as one explanation for the higher cyst numbers occurring outside an affected centre. Further, it is quite probable, as has often been suggested, that "eelworm disease" may be due to an association of the fungus *Rhizoctonia solani* with *Heterodera schachtii*.

The authors would like to express their thanks to Messrs I. S. Macdonald, H. W. Miles, J. Orr and E. Holmes Smith, of the Agricultural Advisory Department, for assistance in collecting the records on crop failure, locating diseased areas and making the soil samples, and to Mr L. Tippet for advice in the statistical treatment of the results.

### SUMMARY.

1. *Heterodera schachtii* has been identified in many parts of Lancashire and Cheshire where severe losses have resulted from failure of the potato crop.
2. A detailed study of the errors involved in the technique of a method for estimating the infestation has been made.
3. The degree of infestation, as determined by cyst counts, has been compared with records of crop failure.
4. The conclusions that have been drawn are to the effect that: (a) where "eelworm disease" has been noted recently there is a positive association of intensity of disease and cyst content of the soil; (b) where the disease was observed three or four years ago, disease and crop failure are always associated with a high cyst content of the soil, but there may be a moderately high infestation without apparent diminution of crop yield.

## REFERENCES.

- (1) FISHER, R. A., THORNTON, H. G. and MACKENZIE, W. A. (1922). The Accuracy of the plating method of Estimating the Density of Bacterial Population. *Ann. App. Biol.* 1x, 325.
- (2) FISHER, R. A. (1928). *Statistical Methods for Research Workers*. 2nd ed. Oliver and Boyd.
- (3) KÜHN, J. (1881). *Die Ergebnisse der Versuche zur Ermittlung der Ursache der Rübenmudigkeit und zur Erforschung der Natur der Nematoden*. Dresden.
- (4) MORGAN, D. O. (1925). Investigations on Eelworm in potatoes in South Lincolnshire. *Journ. Helm.* III, 185.
- (5) STRUBELL, A. (1888). Untersuchungen über den Bau und die Entwicklung des Rubennematoden *Heterodera schachtii*. *Bibliotheca Zoologica*, H. II, p. 1.
- (6) ZIMMERMANN, H. (1920). Nematodenbefall (Heterodera) an Kartoffeln. *Zeits. f. Pflanzenkrankheiten*, Bd. xxx, p. 139.

(Received October 30th, 1928.)

## INVESTIGATIONS ON *HETERODERA SCHACHTII* IN LANCASHIRE AND CHESHIRE

### PART II. THE RELATIONSHIPS BETWEEN DEGREE OF INFESTATION AND HYGROSCOPIC MOISTURE, LOSS ON IGNITION AND pH VALUE OF THE SOIL

By A. M. SMITH, B.Sc., Ph.D., A.I.C.

(*Adviser in Agricultural Chemistry, Manchester University.*)

(With 2 Text-figures.)

It has been shown (Part I) that an infestation of *Heterodera schachtii* may not be uniform over even a small area. It seemed desirable, therefore, to make an examination of certain soil characteristics to see if any relationship existed between the degree of infestation and the nature of the habitat. It was thought that the rate of reproduction of the nematode might be influenced by moisture and temperature conditions. Hence, attention was first directed to those physico-chemical properties which are directly related to soil moisture and temperature.

#### MOISTURE AND LOSS ON IGNITION.

Keen and Russell(3) have produced data which show qualitatively that the moisture variations in a soil are inversely related to the mean temperature; in other words, the soil warms as it dries and *vice versa*, and a rapid rise of temperature, in spring for example, does not occur until the soil has lost its excess of moisture. In another investigation on single value determinations, Keen and Coutts(4) have shown that a good correlation exists on the one hand between content of organic matter and the moisture at the "sticky point," and on the other hand, between clay content and the equilibrium moisture content at 50 per cent. humidity. The last mentioned is in turn closely related to "air dry moisture." The soils in the present investigation were either peaty sands or peats and it seemed, therefore, that the simplest method of obtaining comparative figures for field conditions of moisture and temperature was to determine the moisture content and the loss on ignition of the air-dried soil.

All the samples concerned were made in the course of a few days,



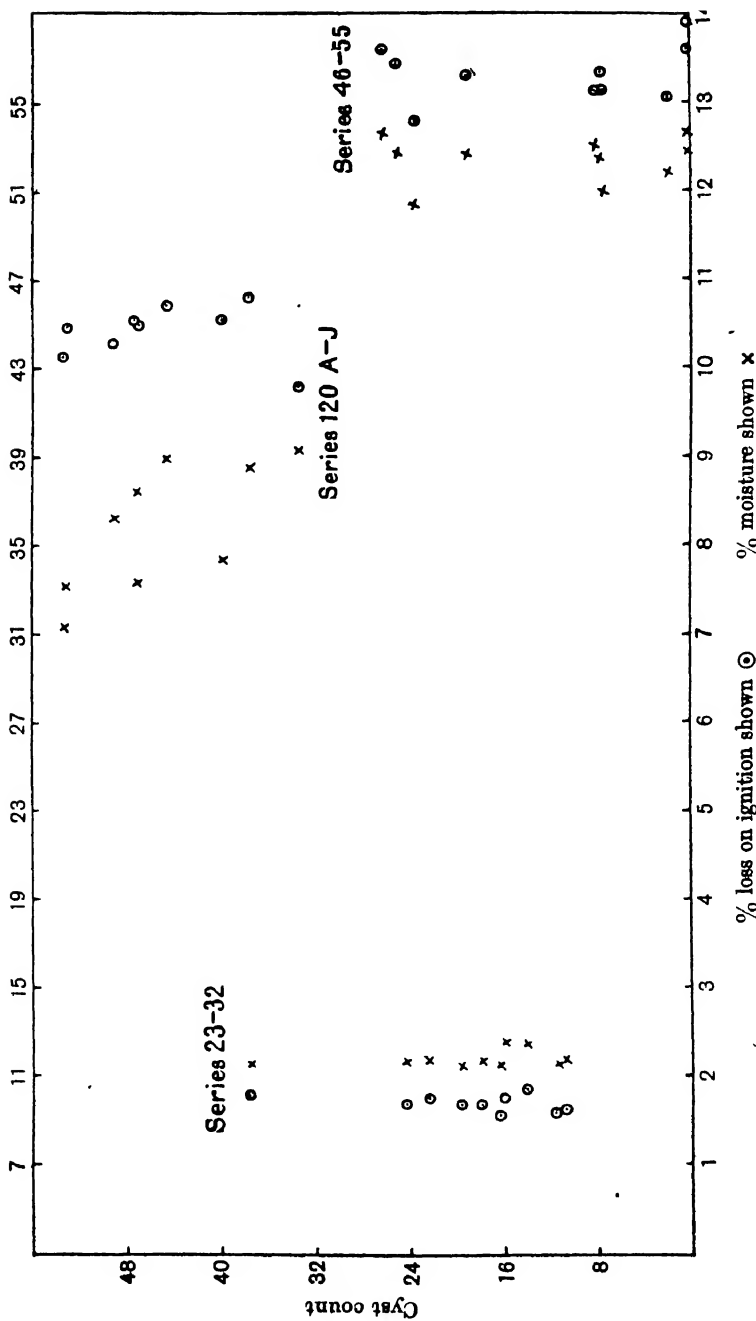


Fig. 1. Diagram showing relationship between infestation of *H. schachtii* and moisture, and loss on ignition for three series of soil samples.

while the samples of any one series were taken in one day. The air-dried and sieved (2 mm.) soil was employed. The usual procedure was to heat the soil to a temperature of 100–105° C. for about five hours, weigh and then ignite to constant weight. The conditions were identical for each series and the determinations were made in duplicate. Values were obtained for three series in each of which there was a wide and significant variation in cyst content.

The values for both moisture and loss on ignition are, however, quite scattered and uncorrelated with the cyst counts (Fig. 1). It is almost certain that characteristics arising from excessive dryness on the one hand, or bad drainage and excessive moisture on the other hand, would have been revealed by those measurements. Since those series were typical of the different field conditions met with in the course of the investigation it seemed useless to pursue this line of study further.

#### ACIDITY.

Another soil property upon which a vast amount of work has been carried out, and the determination of which provides a useful figure in the consideration of acidity and base exchange problems, is the *pH* value. It gives a measure of the hydrogen ion concentration of a soil or of, what is usually determined, an aqueous suspension of the soil. An investigation on the relationship between *pH* and cyst counts was carried out by Peters (5), who made the measurements colorimetrically on water extracts of the soil. The bulk of his observations refer to one experimental field where the *pH* ranged from about 6 to 6·7, and the cyst content from about 3 to 80 per 10 c.c. of soil. From those results he concluded that there was "an indubitable correlation between *pH* and cyst-concentration" but that a set of miscellaneous samples confirmed that correlation only in part, the reverse sometimes being found.

The *pH* of the soil samples was determined electrometrically by means of the quinhydrone electrode (1, 2). The technique was as follows. About 10 gm. of the sieved soil was shaken vigorously for one minute with about 25 c.c. water and a few decigrammes of quinhydrone. After standing for a few minutes, connection was made with a standard quinhydrone electrode and the potential difference observed. The instrument employed permitted the readings to be made to one millivolt (roughly equivalent to 0·02 *pH*). The standard error was about  $\pm 0\cdot02$  and the results have been given to two places of decimals, although it is realised that such accuracy is liable to misconception in an investigation of this nature.

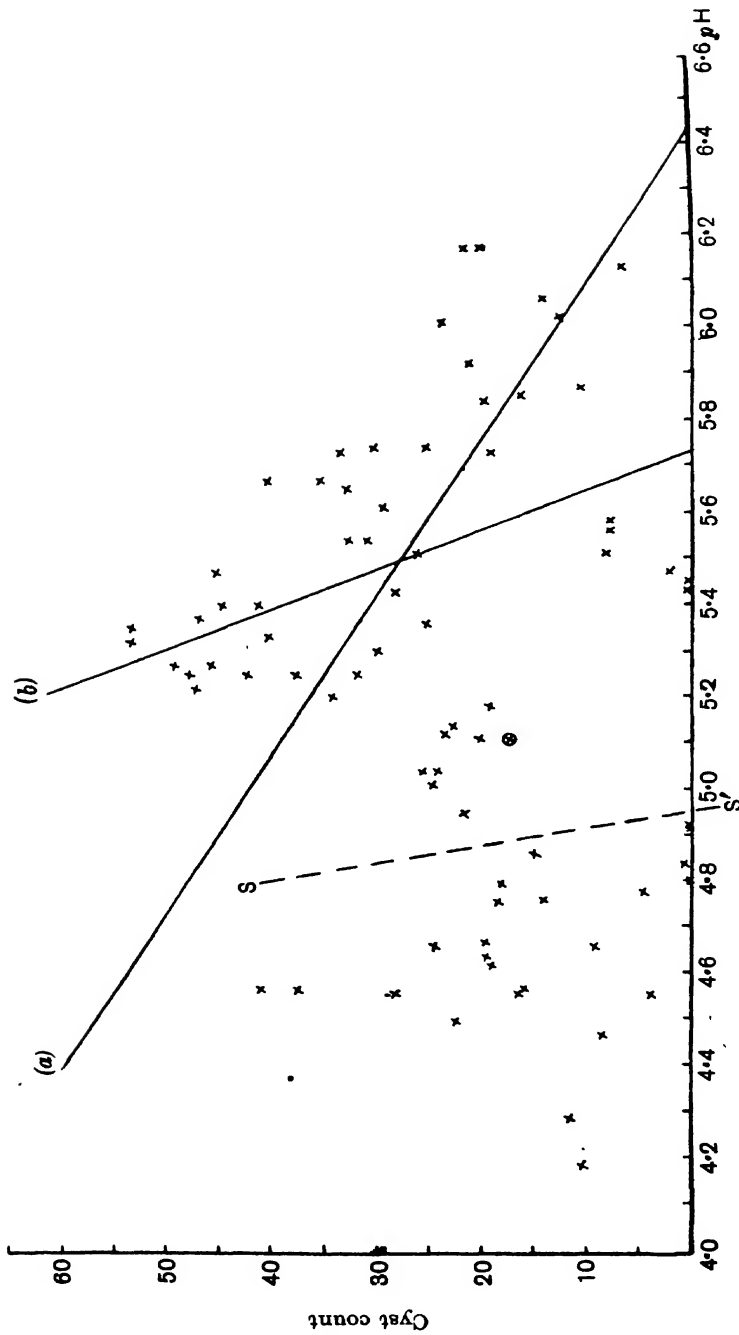


Fig. 2. Diagram showing association between cyst count and pH of soils.

Table I.

*Cyst counts and pH values of soil samples.*

Series 23-32		Series 124 A-124 E		Series 66-75		Series 46-55		Series 113 A-113 C		Series 112 A-112 D	
c.c.	pH	c.c.	pH	c.c.	pH	c.c.	pH	c.c.	pH	c.c.	pH
16.3	4.56	17.0	5.11	8.4	4.47	23.5	6.01	21.0	5.92	21.7	6.17
14.0	4.76	14.8	4.86	3.8	4.56	26.1	5.51	25.0	5.74	19.7	5.84
11.4	4.29	19.7	4.67	27.9	4.56	24.9	5.36	29.2	5.61	16.0	5.85
10.6	4.19	18.6	4.76	40.8	4.57	7.4	5.56			6.6	6.13
15.9	4.57	9.0	4.66	19.0	4.62	19.0	5.73				
22.3	4.50			4.3	4.78	7.6	5.58				
17.8	4.80			0.6	4.84	8.1	5.51				
19.5	4.64			0.1	4.92	1.9	5.47				
24.3	4.66			0.1	4.92	0.3	5.43				
37.5	4.57			none	4.80	0.2	5.45				

Series 11-20		Series 111 A-111 D		Series 6, 10		Series 56-65		Series 120 A-120 J	
c.c.	pH	c.c.	pH	c.c.	pH	c.c.	pH	c.c.	pH
46.7	5.37	12.5	6.02	29.7	5.30	45.7	5.27	33.3	5.73
41.2	5.40	10.6	5.87	42.0	5.25	33.8	5.20	44.5	5.40
30.5	5.54	20.1	6.17			21.4	4.95	37.5	5.25
35.0	5.67	13.9	6.06			23.9	5.04	47.3	5.25
32.4	5.65					22.5	5.14	49.1	5.27
32.3	5.54					23.3	5.12	39.8	5.33
27.9	5.43					25.5	5.04	53.1	5.35
30.1	5.74					24.6	5.01	53.3	5.32
44.9	5.47					20.0	5.11	47.0	5.22
40.2	5.67					18.8	5.18	31.3	5.25

The first three series are peaty sands, the remainder are peats.

For the purpose of a preliminary examination the two variables, *pH* and cyst count, were plotted in the form of a dot diagram (Fig. 2). The figures for *pH* range from about 4.2 to 6.2, and those for cyst count from 0 to about 53 per 10 c.c. soil. At first there appeared to exist no simple association unless it were that the maximum concentration occurred in the neighbourhood of *pH* 5.3 and that less cysts were found on either side of that point. The picture, however, is considerably altered when the two soil types—peat and peaty sand—are regarded separately. By a fortunate chance all but one of the peaty sands are more acid than the most acid of the peat soils and the broken line *SS'* effects a simple division of the two groups: a circle has been drawn round the unique point of the smaller group, occurring beyond the line *SS'*. A consideration of the points representing the larger group of peat soils to the right of *SS'*

shows that there appears to be a general trend indicating a negative correlation between pH and cyst count. Mention has already been made of the totally different characters of the two soil types, and the figures of Table II emphasise those differences in physical properties as exemplified by apparent specific gravity. It was decided, therefore, to analyse the results for the two groups separately.

It was further considered advisable to regard all the pairs of observations as one large sample from an area over which the occurrence of *H. schachtii* is widespread. The pH through a strip 100 yards long on a field usually fluctuates to a certain extent owing to a variety of causes, but the range is rather short and ten pairs of observations at most, too limited in this case to secure a satisfactory coefficient of correlation. Taking the 53 pairs of observations as a whole, therefore, the figures for pH vary from 4.95 to 6.17, those for cyst count from 0 to 53.3 per 10 c.c. soil.

If  $X$  = any cyst count and  $Y$  the corresponding pH value,

$x$  = the deviation of any cyst count from the mean  $\bar{X}$  (27.25),

$y$  = the deviation of any pH value from the mean  $\bar{Y}$  (5.50),

then  $r$ , the coefficient of correlation =  $\Sigma xy / \sqrt{\Sigma x^2 \Sigma y^2}$ .

In this case  $r = -0.504$ .

The probability that such a correlation would have been obtained from any random comparison is less than 0.01, so that the correlation is undoubtedly significant. The coefficients of regression (6)

$$b_1 = r \frac{\sigma_x}{\sigma_y}, \quad b_2 = r \frac{\sigma_y}{\sigma_x},$$

where  $\sigma_x$  and  $\sigma_y$ , the standard deviations of cyst counts and pH, are respectively 13.86 and 0.2361, have been calculated:

$$b_1 = -29.59, \quad b_2 = -0.008592.$$

The regression equations are therefore  $x = -29.59 y$ ,

$$y = -0.00859 x,$$

or replacing  $x$  by  $(X - 27.25)$  and  $y$  by  $(Y - 5.50)$

$$X = 190.05 - 29.59 Y \quad (a),$$

$$Y = 5.7342 - 0.00859 X \quad (b).$$

The dot diagram has been completed by inserting the lines representing the equations (a) and (b). An examination of the diagram reveals that the correlation coefficient is reduced by the group of values for the observations 58-65 (see p. 336, Part I) all below pH 5.20 and with comparatively low cyst counts. They appear to belong to another population.

## 346 *Heterodera schachtii* in Lancashire and Cheshire

There is little doubt, therefore, that some correlation, worthy of further investigation, exists between cyst count and pH, but that there are other factors at play.

When the twenty-five pairs of observations for the sandy soils are subjected to the same treatment, the coefficient of correlation is found to be  $-0.176$  which is certainly not significant.

Table II.

*Apparent specific gravities of two soil types.*

Type	Peaty sand		Peat soil				
Sample	Composite	124 B	111 A	112 A	113 A	120 A	120 I
Apparent spec. grav.	1.20	1.23	0.55	0.61	0.54	0.61	0.54

### SUMMARY.

1. Soil samples taken for the purpose of measuring the infestation of *Heterodera schachtii* have been further examined for any association between cyst concentration and those physico-chemical properties relating to the moisture, temperature and acidity of the soil.

2. The hygroscopic moisture and loss on ignition have been determined for three typical series of ten samples having significant variations in cyst counts. There is no simple association of the observations within any of the series. It appears improbable, therefore, that the rate of reproduction of *Heterodera schachtii* is influenced to a marked degree by the normal variations in the physical condition of the soil.

3. The pH of seventy-eight samples has been measured electrometrically. When the samples are divided into two groups according to soil type, there is found to be a significant negative correlation between pH and cyst count for fifty-three peat soils. There is not, however, a significant correlation in the case of the other twenty-five sandy soils.

### REFERENCES.

- (1) BILMANN, E. (1924). On the Measurement of Hydrogen-Ion Concentrations in Soil by Means of the Quinhydrone Electrode. *Journ. Agric. Sci.* xiv, 232.
- (2) BILMANN, E. and TOVBORG-JENSEN, S. (1927). On the Determination of the Reaction of Soils by Means of the Quinhydrone Electrode. *Trans. 2nd Comm. Intern. Soc. Soil Sci.* vol. B, p. 236.
- (3) KEEN, B. A. and RUSSELL, E. J. (1921). The Factors Determining Soil Temperature. *Journ. Agric. Sci.* xi, 211.
- (4) KEEN, B. A. and COUTTS, J. R. H. (1928). "Single Value" Soil Properties. *Journ. Agric. Sci.* xviii, 740.
- (5) PETERS, B. G. (1926). *Heterodera schachtii* and Soil Acidity. *Journ. Helm.* iv, 87.
- (6) YULE, G. U. (1927). *An Introduction to the Theory of Statistics*. 8th ed. Griffin and Co. Chap. ix.

(Received October 30th, 1928.)

## REVIEWS

*Physiology and Biochemistry of Bacteria.* Vol. I. Growth Phases; Composition and Biophysical Chemistry of Bacteria and their Environment, and Energetics. By R. E. BUCHANAN and E. I. FULMER. Pp. xi + 516; 78 Figs. London: Baillière, Tindall & Cox. 1928. \$ 7.50.

Bacteriology has for long been merely the handmaiden of Pathology and Industry and a Science of Bacteriology can at present hardly be said to exist. A vast amount of empirical data has been accumulated but this is scattered in almost heart-breaking fashion through journals devoted to agriculture, general biology, medicine, chemistry, physics and various industrial applications and the need is perhaps peculiarly urgent for the compilation and systematisation of this material.

Chapter I of the present volume, consisting of a little over two pages, is devoted to a consideration of the scope of physiological bacteriology. It gives what is really the keynote of the work and is summarised in the statement that 'physiological bacteriology includes all the applications of mathematics, physiology, chemistry, and physics to the problems of the life and growth of micro-organisms.' In support of this comprehensive claim the authors, in later chapters, make wide ranging excursions into territories which are not usually regarded as pertaining to bacteriology. Their wide net sweeps up not only bacteria, but fungi, slime molds, protozoa and even algae and the reader is led into mazes of physics, chemistry and mathematics which are apt to confuse the essential issues. The embracing character of the work may be gauged from a skeleton outline of its contents.

Chapter II deals with the growth phases and growth rates of micro-organisms in culture. Following a review of the various methods, direct and indirect, of estimating numbers and amounts of growth in culture, there is a discussion of the actual growth phases of various kinds of micro-organism and a consideration of growth itself as an autocatakinetic phenomenon. In relation to this field of study the authors sound a much needed warning against "interpreting causal relationships on the basis of curves plotted or the equations which describe them."

In Chapter III a discussion of microscopic and macroscopic methods of analysing the chemical composition of the cells of micro-organisms is followed by a lengthy consideration of the actual data of such analyses.

Chapter IV, containing 234 pages, is really a general treatise on physical-chemistry and in it are considered at length the nature of true solutions and of colloidal solutions and the colloidal state, and more briefly, such questions as refractivity, specific gravity, diffusion and conductivity. In discussing electricity as a tool in measurement the authors emphasise a point of view that might perhaps be more seriously considered by many teaching-schools of biology, viz. that "No biologist can be considered as equipped with a full kit of tools for the exploration of physiological process who is not conversant with the fundamentals, not only of electricity in general, but more especially of the behaviour of the cell, tissue, or tissue-fluid toward the electric current."

In Chapter V are considered the energy relationships, growth and movement of micro-organisms. After brief reference to the units of force, work and power, the chapter is mainly devoted to a discussion of the types and sources of energy for micro-organisms and the various ends—synthesis, heat, light and movement—to which they are utilised.

The volume closes with 30 pages of "literature cited," author and subject indices and an index to names of micro-organisms in the text.

Volume II, which has not yet appeared, will be concerned with the influence of environmental conditions on microbial development, with changes produced by bacteria and with the mechanism of such changes.

It will be obvious that the work is planned on rather an epic scale and one must admire the authors' courage in setting out on such a task and be grateful for what they have already given us. One may be accused of looking a gift horse in the mouth but one cannot help feeling that the book might possibly have been more useful and successful if the authors had been content with a lesser ambition. After all, there are within easy reach excellent text-books of physiology, physics, chemistry, physical chemistry and mathematics and there seems little reason why this much needed volume on *The Physiology and Biochemistry of Bacteria* should be ballasted with so many of the fundamental teachings of these subjects. No one denies their importance in the nascent science of bacteriology, but their detailed exposition seems out of place as an integral part of a treatise of this nature and the resultant clogging of the more direct issues tends to induce a state of mental indigestion on the reader's part. On the other hand, it is undoubtedly convenient to have gathered together in a single treatise the relevant portions of so many diverse sciences and as the volume is "essentially a combination and revision of the lectures on physiology of bacteria and on biophysical chemistry given at the Iowa State College to students of bacteriology", one assumes that in practice this convenience has been found by the authors and their students to be so great as to outweigh other considerations.

As a book of reference the work is of a very considerable value. It is clearly and simply written and it incorporates into a logical scheme a great mass of data many of which would otherwise not easily be accessible. The standpoint of the authors is fair and judicious and where necessary both sides of a case are presented. There can be no doubt that the volume will fulfil the hope that it "may call forcibly to the attention of students the wide gaps that exist in our knowledge of the physiology of micro-organisms, and stimulate additional research in this field." It is perhaps characteristic of the viewpoint throughout that the work ends with the sentence "An explanation is not forthcoming!"

WILLIAM B. BRIERLEY.

*Life in Inland Waters: with especial reference to animals.* By KATHLEEN E. CARPENTER. With an Introduction by JULIAN S. HUXLEY. Pp. xviii + 267. London: Sidgwick and Jackson, Ltd. 1928. 12s. net.

The necessity for the provision in this country of facilities for the investigation of problems of fresh-water biology is being increasingly recognised and we have rather suddenly awakened to the fact that in the scientific study of our inland waters we lag considerably behind many other European countries. The general position to-day is, in fact, little different from that pictured by Kofoid in 1910 in his treatise on *The Biological Stations of Europe*. For many years well equipped limnological institutes have been in active existence in Russia, Germany, Austria, Sweden, Denmark, Belgium and other countries, whilst in England our only centre has been a small private laboratory established in 1901 by Mr Eustace Gurney on Sutton Broad. English investigations have been carried out by the Wests, the Pearsalls and other individuals mostly working from a University centre and carrying with them into the field such facilities as they could provide. More recently the Ministry of Agriculture and Fisheries have conducted investigations into the ecology of streams and considerable extension of this work is imminent.

The subject was brought to the front by Professor Fritsch who, in his Presidential Address to Section "K" of the British Association in 1927, emphasised the urgent need for the establishment of an active and well equipped fresh-water biological station in Great Britain. A further step was taken the following year when at Glasgow, Sections "D" and "K" held a joint discussion on the subject which resulted in the appointment of a small Committee to consider the means to be adopted for the



establishment of such a station. The Committee has just issued a report and it is greatly to be hoped that its recommendations will lead to the establishment of the suggested station in the English Lake District.

Meanwhile, by the publication of her admirable book, *Life in Inland Waters*, Dr Carpenter has most opportunely provided a text which should do much to help forward this very necessary development. The book is no dry-as-dust academic compilation but an interestingly written survey of the animal ecology of fresh waters and it has caught the very spirit of that fascination of "pond-life" which in our teens lured so many of us into biological ways.

Following an introductory chapter on the general characteristics of the fauna of inland waters, Chapter II deals with the components of the fauna and their general activities and interrelationships, and Chapter III with their relationships to the chemical and physical factors of the habitat. Chapter IV, dealing with the reproduction of fresh-water animals, is one of the least satisfactory portions of the book, but this is almost inevitable, for the author can only touch the surface of so thrilling and formidable a subject in the few pages allowable and this she has done extremely well. Chapter V is a tantalisingly brief consideration of the geographical distribution and dispersal of fresh-water animals and of the influence of the great ice age in Europe. The next four chapters deal with the special features of particular kinds of habitat and show how far these special features influence the life which characterises them. In two of these chapters we are taken from head streams and highland brooks along the minnow reaches and finally arrive at the lower reaches and estuarine waters. In the following two chapters are discussed the biology of lakes and of small or peculiar water bodies such as marsh and moorland waters, cold and hot springs, subterranean waters, etc. The last and perhaps most interesting chapter deals with the biology of inland waters in relation to human life and considers among other questions the vexed and urgent problems of the pollution of rivers by town drainage and industrial wastes.

The chapters are headed with delightfully apt quotations from Isaac Walton, that most lovable father of fresh-water biology, and terminated by very useful bibliographies that cover an unusually wide range of literature. There are good subject and author indices and the book is excellently illustrated by twelve plates and 94 text figures. The volume is one of the series of text-book of Animal Biology edited by Professor Julian Huxley, its format is very pleasant and as modern prices go it is good value for the money.

A volume of this size dealing with so comprehensive a subject must obviously be a little superficial, and yet on reading the book one is left rather with the impression that most problems of any importance have been at least indicated. Further, in spite of its size and interest the book contains an astonishing amount of data. Breathing new life into a subject that academic minds and laboratory researches have almost killed, this is a book that one would like to put into the hands of all undergraduate students of biology. There are few of us who, even now, do not like to dabble surreptitiously in "pond-life", and undergraduate students for whom "pond-life" is legitimate will find in Dr Carpenter's book the guide they need.

WILLIAM B. BRIERLEY.

*The Scientific Principles of Plant Protection.* By HUBERT MARTIN, with a Foreword by Sir DANIEL HALL. 316 pages. London: Edward Arnold. 1928. 21s.

A cordial welcome may be offered to this book, which should prove a valuable contribution to a subject of growing importance. Sir Daniel Hall remarks in his foreword: "There is a general theory of plant medicine or plant hygiene and Mr Martin's book is the first introduction to it which has appeared in English." It is certainly true that most previous works have treated the subject of "Plant Protection" primarily from the botanical or zoological aspects, and it is all to the good, therefore, that a

chemist, such as Mr Martin, should intervene in a field so largely occupied by entomologists or mycologists, and summarise the facts bearing upon "Plant Protection" from a different standpoint.

Mr Martin's method of dealing with his matter is to divide it into sections and subsections—e.g. **Plant Resistance**, **Insecticides**, and **Contact Insecticides**—and then, after some introductory remarks to each section, to quote or summarise the writings of the various investigators who have contributed information bearing upon the subject under discussion. From the chemical standpoint the literature appears to have been examined minutely: upwards of some 800 references are given to the works of nearly as many writers, and this alone will render the book most useful as a work of reference. Detailed criticism would be out of place because the author is giving not his own conclusions but those of other writers, and where so many investigators are concerned, it is inevitable that the articles quoted should be of unequal merit, a point, however, against which the junior reader might perhaps be warned. A few misstatements, or misinterpretations, will be noted (e.g. p. 4, that nematodes are "true worms with ringed bodies," and p. 245, that the Brown Tail Moth was introduced into the U.S.A. from England) but in the first edition of so comprehensive a compilation occasional mistakes are almost inevitable, and in the present case they do not detract from the usefulness of the whole. One suggestion may perhaps be made, and it is that the title is a little misleading. From the chemical aspect the book is much more than a statement of scientific principles, while from the biological side the title suggests fuller discussion of certain subjects than is actually afforded them. "Plant Protection from the Chemical Standpoint" would adequately describe at least 80 per cent. of the book, and would perhaps give the purchaser a better idea of what to expect. Subject and author indices are provided, and in so far as they have been tested appear to be satisfactory. The author is to be congratulated on having produced a book which will be of value to all concerned with "Plant Protection."

J. C. F. FRYER.

*Spraying, Dusting and Fumigating of Plants.* By A. FREEMAN MASON.  
539 pages, 237 Figs. New York: The MacMillan Co. 1928. 21s.

This book is the most recent of the series of Rural Manuals edited by L. H. Bailey, a series which needs no introduction to English readers. The present volume resembles its predecessors in being practical and comprehensive, well printed and adequately illustrated: in fact, it is typical of American books of its class, and the opportunity may be taken of expressing admiration for the gift possessed by the American writer of rendering the results of scientific investigation available to the "practical man," although it must be admitted that this task is facilitated by the readiness of the American reader to digest a fair amount of technical matter if a commercial advantage can be secured.

In regard to Mr Mason's book, it may first be pointed out that whereas the title adequately describes the first half of the book, the second half—comprising something like 250 pages—is better indicated by the sub-title "a popular Handbook on Crop Protection," since it comprises a popular account of the more important American plant pests and diseases, with measures for dealing with them, not only by spraying, dusting, or fumigation, but by other means as well. This latter half of the book is necessarily of less interest than the first to the English reader, who for information as to American pests and diseases will rather refer to such works as Hesler and Whetzel's *Manual of Fruit Diseases*, or the similar work on Fruit Insects by Slingerland and Crosby: even so, it may be suggested that in a subsequent edition the scientific names of the various pests and diseases dealt with might be inserted, an omission seldom noted in an American work.

Turning to the sections of more general interest, Chapters I-V give some account of the history of spraying, the principles underlying spraying practices, and of the

insecticides and fungicides in general use. These chapters form a useful and convenient summary of the modern American viewpoint on these matters. It is a little odd, however, to find the attractiveness of geraniol to the Japanese Beetle dealt with on p. 26 under "Repellents," and the statements on pyrethrum on p. 66 are hardly in accord with the most recent work on the subject. English apple growers will read with envy on p. 50 that the American Apple Capsid can be controlled by the use of a nicotine spray containing only about 0.025 per cent. pure nicotine—half the strength required in spraying against the English Apple Capsid (*Plesiocoris rugicollis*).

Chapters VI to IX, dealing with "Selecting the Spraying Machine," "Qualifications of Spray Machinery," the "Central Stationary Spraying Plant," and the "Art of Spraying," form decidedly the most interesting part of the book, and should be read by all who are concerned with spraying whether from the research or commercial points of view. Specially worth attention are the remarks of the author upon the need for sufficient power in spraying apparatus. The smallest engine-driven sprayer which he considers of value is one with a single cylinder pump worked by a 1½–2 h.p. engine, with an average output of 3–4 gallons per minute at 250 lbs. pressure, and he emphasises that this will only supply one spray rod with two disc nozzles or one spray gun with a small orifice in the disc. This apparatus he considers useful for a 5-acre orchard. For spraying a 25-acre plantation he advocates a 3-cylinder pump and an engine of from 3½–6 h.p., and even so he gives a warning that unless the machine is at its best it will not satisfactorily supply more than one gun with a large aperture. This insistence on adequate power as the first essential in spraying machinery occurs throughout the chapters concerned, and is specially mentioned as it is well worth English attention. Of almost equal interest is the need for apparatus which will do the work sufficiently quickly—a need which is strongly emphasised by the author. He believes a satisfactory spraying equipment should be able to cover the whole area to be sprayed in four days, because on an average there are not more than four days fit for spraying in any week. In England this estimate would often prove too optimistic, and therefore the need for quick work is all the more imperative. Chapter VIII on the "Central Stationary Plant" shows that this method of dealing with the spraying problem, a method in which England is the pioneer, is now attracting considerably more attention in the U.S.A. Finally, reference may be made to Chapter X—"Dusts and Dusting," the author's considered opinion on this debatable subject being that "The day for unequivocal recommendations of dusting is not yet at hand but indications are that it will soon dawn. Until then the orchardist and gardener must consider the liquid sprays the standard materials." In this chapter some reference is made to dusting machinery, but it is relatively slight as compared with the descriptions of spraying apparatus given in the earlier chapters: in a subsequent edition a full discussion of the mechanics of dusting apparatus would be welcome.

On the whole the book, although written for the American "orchardist" and "truck crop" grower, has much in it of value to the English reader, and it is to be commended not only to the notice of the practical grower but also to that of the scientific investigator who might well devote more attention to the mechanical and engineering problems involved in pest control.

J. C. F. FRYER.

*Praktische Einführung in die Morphologie der Insekten.* By E. HANDSCHIN.

Pp. viii + 112 + atlas of 23 plates. Berlin: Gebrüder Borntraeger.  
1928. (Sammlung naturwissenschaftlicher Praktika, Bd. 16.) 11 R.M.  
cloth bd.

Notwithstanding the numerous text-books dealing with diverse aspects of entomology, both theoretical and applied, that have appeared during the last few years they leave the laboratory training of the student only scantily provided for. Practical

guides to the dissection and study of insects are so few and far between that Schoenichen's "Praktikum der Insektenkunde" (2nd edition) and Comstock and Needham's "Elements of Insect Anatomy" (10th edition) are the only works that can be recalled as being in any way comparable with the new book by Dr Handschin. In his *Praktische Einführung*, Dr Handschin has provided a type of manual that is particularly welcome and can be recommended as a thoroughly sound and reliable introduction to the elements of insect morphology. Throughout its pages he interprets structure in relation to function and having mastered the course thus planned for him, the student should be sufficiently equipped to understand the significance of the chief modifications that insects undergo. He should, for example, be able to deduce how a given insect lives, how it obtains its food and what kind of food it is dependent upon.

Dr Handschin's method of treatment is to divide the book into chapters, each dealing with a separate region of the insect body, and he utilises various types of insects in illustration of the diverse modifications the organ or region in question may undergo. The introductory chapter is concerned with (a) methods of preserving, fixing and mounting specimens; (b) a list of the principal works on general entomology useful to the student; and (c) an enumeration of the different species of insects required in order to follow the full course laid down. The succeeding chapters deal as follows: (I) with the chitinous skeleton; (II) the head; (III) the head appendages; (IV) the thorax; (V) the abdomen; (VI) the endoskeleton; (VII) auditory and chordotonal organs; and (VIII) the spiracles. Most of the chapters are further subdivided into sections or lessons and as an example of Dr Handschin's scheme of instructions section B—the mouth-parts, of Chapter III may be selected. The section is preceded by a list of papers deemed useful to the student in his work: the list is short enough to avoid confusing a beginner and includes some of the important papers written in English and continental languages. There follows an enumeration of various common insects necessary for the student to have at hand in order to carry out the prescribed work on mouth-parts. The introductory type adopted is *Periplaneta*, which is used to explain the general plan of the insect mouth-parts and as an example of an insect with omnivorous feeding habits. The carnivorous type, as illustrated by *Cicindela* follows next, and the adaptations to a flesh-eating habit are clearly stressed. The herbivorous type is exemplified in the cockchafer and then there follows a short discussion of certain more special types of trophi as is seen in *Collem-bola*, *Dytiscus*, larvae, Odonata, etc. The student is next introduced to the nectar-sucking types of mouth-parts, Lepidoptera being utilised to illustrate the evolution of the haustellum, and various common Hymenoptera to demonstrate the part played by the ligula in sucking and "licking." The highly specialised piercing mouth-parts are considered under two categories. Firstly the blood-sucking type, and here the student is required to study a Culicid, followed by a Tabanid and finally *Stomoxys* and *Glossina*. Secondly the plant-sucking type which is conveniently illustrated by *Pentatomia* or *Graphosoma*. The sixth type of mouth-parts is the highly evolved suctorial and licking arrangement seen in the proboscis of various Diptera, *Musca* or *Calliphora* providing the necessary material.

As a further guide to the prescribed course an atlas of 23 plates is provided in a pocket at the end of the book. Here the student will find exceptionally clear figures illustrating practically every feature in insect structure that is dealt with in the text. The features which they portray are all taken from common and easily obtainable insects, most of which the student can collect for himself in almost any good locality.

By way of constructive criticism we make the suggestion that in the event of a second edition of the book being called for, as we think will happen, the author would be well advised to consider the inclusion of certain features of internal anatomy. Chapters on the respiratory, digestive and reproductive systems and their chief modifications would be valuable and not unduly enlarge the book. There is happily very little by way of adverse criticism that calls for mention. Typographical errors are few and these mostly have been noted and listed by the author on p. viii. On p. 35 Cantharididae is evidently meant for Cantharidae while throughout the book *Stomoxys* is spelled *Stomoxis* and *Lymantria* is spelled *Limantria* except on p. 9.

These, however, are very minor blemishes in a thoroughly sound elementary handbook.

A. D. IMMS.

*The Problems of Applied Entomology.* By ROBERT A. WARDLE. Manchester University Press. Biological Series, No. V. 1928. Pp. xii + 587; frontispiece and 31 illustrations. 30s. net.

The author of this book, in conjunction with the late P. J. Buckle, published a work of a very similar character in 1923, entitled *Principles of Insect Control*. The latter volume was the first ever written dealing in a comprehensive manner with the multifarious aspects of the subject of insect control, and it was well received both in this country and in America. The present volume is to be regarded as supplementary to its predecessor and, to a large extent, only takes cognisance of work that has appeared since 1922: it is a considerably longer book with the subject matter arranged on a somewhat different scheme.

Part I, which comprises 247 pages, is devoted to General Problems, and under this heading the many different methods and factors that exercise a controlling influence on noxious insects are discussed. In these pages the reader will find a clear account of some of the most recent additions to knowledge of that wide range of subjects. Mention may be made of Uvarov's important theory of migratory and non-migratory phases in locusts; the immensely important subject of virus disease transmission; the insect control of weeds; chemotropism; biological control by parasites and predators; and an extended discussion on insecticides.

Part II, comprising 271 pages, discusses "Area Problems", and this section of the book breaks new ground, in that the author here gives a concise account of the problems applied entomologists have to face in different regions of the globe, the major pests that prevail there, and the "local" controlling measures in force. It brings together much scattered information not easy of access, and has evidently been the result of industrious search through a very large number of departmental bulletins, annual reports and circulars. The two concluding chapters of the section are headed "Locality Disinfection" and "Locality Protection." The expression locality is used in a somewhat vague implication since it may refer to the soil around the roots of a particular plant, or to a bale of cotton, a particular field or even to an extensive tract of country. By "Disinfection" is meant the destruction of noxious insects, and in the chapter "Locality Disinfection" three main problems are considered, viz. the destruction of insects affecting animals or plants (a) before the latter leave the locality or area wherein they have been reared, and (b) before or immediately after their admission into a new area, or at least before they become widely dispersed, while (c) discusses the eradication from a restricted locality of an introduced insect of known potentiality. Sections (a) and (b) deal with schemes which lie outside the more usual methods of insect control, in that they aim at the absolute eradication of the insects present, instead of reducing an infestation to a degree compatible with successful practice. Section (c) also differs from ordinary practice in that it does not take cognisance of control measures in relation to the market value of the product infested. It only considers the question of eradicating a dangerous insect, irrespective of cost. The chapter on "Locality Protection" is largely concerned with such legislative measures as embargoes and quarantines.

Part III (67 pages) is occupied with a classified bibliography, an index of authors referred to and a subject index: the bibliography is not intended to be complete, but it lists the representative economic literature of the past seven years, and judicious selection seems to have been exercised.

A book which embraces so wide a field usually presents some features wherein a reviewer may find himself not in complete agreement with the author. In the volume before us insecticides come in for a very large share of treatment: one would have liked to have seen a few of these pages devoted to a more extended discussion of the

growing subject of virus diseases and their insect transmission, which are so much in the minds of plant pathologists to-day. As a further suggestion, the section on biological control would repay extending. This method is attracting attention in almost every country of the world, but we are as yet only on the very fringe of the possibilities attending its application and the pitfalls are many. A fuller discussion of the principles involved, the possible causes of success and failure in specific cases, and some account of the technique of mass rearing of parasites would be a welcome improvement. These few points we hope may receive consideration in the event of a third volume of similar scope being contemplated.

Books of the type of the present one are becoming more and more necessary in all subjects, like applied entomology, whose advances are accompanied by such an enormous output of diverse kinds of literature. It is of the greatest assistance to the investigator, the teacher and the advanced student to have access to a work which takes stock of the present day position of the subject concerned. This fact Prof. Wardle has evidently recognised to the fullest degree and we congratulate him upon the production of a well written, well arranged and, it might be added, almost indispensable volume.

The publishers are to be complimented on the printing and general "get up" of the book, but it is a matter of disappointment that they have found it necessary to fix its price at so relatively high a figure.

A. D. IMMS.

## REPORT OF THE COUNCIL OF THE ASSOCIATION OF ECONOMIC BIOLOGISTS FOR THE YEAR 1928

DURING 1928 the Association has met on eight occasions. At five of these, various subjects of interest were brought before the Association by members and visitors to whom it is greatly indebted. The subjects included Marine Biology, Forest Products Research, Biology of Wood Wasps, Physiology of Plant Disease, and Preservation of Timber. At the Annual General Meeting the Presidential Address was given on Legislation in England against Diseases and Pests of Plants, which has been published in Vol. xv, No. 2 of the *Annals* of the Association.

In May, the Association had the privilege of visiting the Royal Botanic Gardens at Kew by kind invitation of the Director, and the summer meeting was held at Bristol and Long Ashton Research Station by courtesy of Professor Barker, the Director.

The first Annual Dinner was held on January 25th.

The attendance at meetings has varied from 39 to 57, with an average of 42. The proportion of members to visitors has been about 10 per cent.

During the year the Association has lost one of its valued members by the death of Dr W. G. Smith.

Two Honorary Members, Professor August Chevalier of Paris and Professor Filippo Silvestri of Naples were elected.

Thirty-three new members have been elected during the year; there have been two resignations and the Association now numbers 283 Honorary and Ordinary Members.

In view of the increase in sales of the *Annals of Applied Biology* it has been necessary to increase the number of copies printed by 100.

During the past year the Association has again enjoyed the hospitality of the Botany Department of the Imperial College of Science and Technology for their meetings. The Council feel sure that the Association will approve of recording its grateful thanks for this privilege.

Papers read to the Association during the year 1928:

*Jan. 20th.* Presidential Address: "Legislation in England against Diseases and Pests of Plants."

*Feb. 24th.* Mr F. A. PANTIN: "The Work of the Plymouth Marine Laboratory."

*March 23rd.* Mr R. S. PEARSON and Mr J. F. MARTLEY: "On the Work of the Forest Products Research Laboratory."

*Oct. 26th.* Mr R. N. CHRYSTAL on "Sirex and its Parasites" and Mr H. ST J. CARTWRIGHT on "A fungus symbiont associated with *Sirex cyaneus*."

*Nov. 23rd.* Mr R. H. STOUGHTON on "The Relation of Environmental Conditions to Angular Leaf Spot Disease of Cotton." Dr W. F. BEWLEY on "The Effect of Environmental Factors on Diseases under Glass," and Mr T. SMALL on "Temperature and Humidity in Relation to *Cladosporium fulvum*."

*Dec. 14th.* Professor PERCY GROOM: "The Antiseptic Preservation of Wood."

## REPORT OF THE HON. TREASURER FOR THE YEAR 1928

During the year 1928 current subscriptions received amounted to £272. 16s. 0d., an increase of £22. 6s. 4d. over the previous year. Arrears of subscriptions amounting to £25. 0s. 0d. were paid while subscriptions considered good, but as yet unpaid, totalled £25. 10s. 0d.; the latter amount compares very favourably with the corresponding figure of £37. 15s. 0d. for 1927. The working expenses of the Association have been considerably less than those of the previous year which is entirely due to a reduction of £252. 19s. 3d. in the publishers' account for the *Annals of Applied Biology*, owing to very material increases in sales of back volumes and parts and of reprints of special articles. After net receipts for sales had been deducted the sum of £175. 16s. 5d. only was required to meet the publishers' charges. It is satisfactory to note that we close the year with a satisfactory cash balance while the incurred liabilities amount to £418. 12s. 4d. of which £388. 7s. 2d. is represented by the publishers' net charges for the cost of production of Vol. xv of the *Annals of Applied Biology*. The Association also has a reserve fund in Savings Certificates now amounting to £531. 5s. 0d.

A. D. IMMS,  
*Hon. Treasurer.*



# THE ASSOCIATION OF ECONOMIC BIOLOGISTS

INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED 31st DECEMBER 1928										Cr	
EXPENDITURE					INCOME						
<i>To Annals of Applied Biology:</i>					£	s.	d.	£	s.	d.	
Estimated Value of Stock at 1 Jan. 1928					55	3	6		25	0	0
Expenditure during 1928					395	4	8		8	7	6
					450	8	2		272	16	0
<i>Less: Estimated Value of Stock at 31 Dec. 1928</i>					62	10	8				306 3 6
<i>To Printing and Stationery</i>								387	17	6	
<i>To Postage and Cheque Stamps</i>								13	4	5	
<i>To Sundry Out-of-Pocket Expenses of Secretaries and Treasurer</i>								7	1	11	
<i>To Audit Fee Reserve</i>								14	13	2	
<i>To Balance, being Excess of Income over Expenditure</i>								4	4	0	
								17	7	6	
								£444	8	6	£444 8 6
										</	



## THE MOSAIC DISEASE OF THE HOP; GRAFTING EXPERIMENTS, II

By D. MACKENZIE, E. S. SALMON, W. M. WARL  
AND R. WILLIAMS.

(*Mycological Department, South-Eastern Agricultural College, Wye, Kent.*)

THE mosaic disease of the hop was first recorded in this country in 1923 when the main features were described and illustrated (6). Thrupp, in 1927 (12), showed that the disease could be transmitted by means of grafting a susceptible healthy scion on an affected stock, and also that a certain Seedling Variety of hop (ref. no. M 45), raised at Wye College, acted as a "carrier," transmitting the disease to scions of susceptible varieties while not itself being visibly affected. Salmon and Ware have pointed out (11) that the mere contact of a virus-carrying scion with the stock is sufficient to cause the infection of the rootstock; also that the commercial variety Fuggles is completely resistant to the mosaic disease, so that when scions are grafted on a severely affected hop plant, they remain healthy, grow with normal vigour and complete their full seasonal development.

### I. THE PROPENSITY FOR "CARRYING" MOSAIC DISEASE, AMONGST COMMERCIAL AND OTHER VARIETIES OF THE HOP.

#### *Methods of experiment.*

The grafting experiments were carried out by D. Mackenzie<sup>1</sup> in 1927 and by R. Williams<sup>1</sup> in 1928. The general method of grafting used was that described and figured by Salmon and Ware in 1925 (7) and 1928 (10). The following details of the experiments may be mentioned.

1927 (D. Mackenzie). The scions used were about 4 to 6 in. in length and were cut at an internode near the ground level. The lower end of the scion was tapered by two downward cuts about half-an-inch long from just below a node. A corresponding cleft was made by two converging cuts downwards in the stem of the stock plant. The graft, after insertion of the scion, was bound by means of a strip of thin rubber, half-an-inch wide and 5 to 6 in. long, the end being secured by a trace of rubber solution.

<sup>1</sup> This work was carried out with the aid of grants from the Ministry of Agriculture and Fisheries.

The scions were taken either from plants of the commercial varieties Tutsham, Cobbs and Eastwell Golding, all of which are very susceptible to mosaic disease, or from wild hops the susceptibility or resistance of which was unknown. The plants providing scions were growing in pots, with the exception of one plant of Tutsham (R 1/50 *a*) and one of Cobbs (R 1/36 *a*) and three plants of Rodmersham Golding (R 1/81 *a*, R 1/82 *a*, R 1/83 *a*) growing in the Experimental Hop Garden at Wye. Each pot plant was labelled and the reference numbers are given in Table I. All plants from which scions were taken were kept under observation during both 1927 and 1928. During 1927 four pot plants of the Tutsham variety showed symptoms of the mosaic disease, and together with the scions taken, were discarded. Three other pot plants of Tutsham, although never showing definite symptoms of mosaic disease, were regarded as being possibly not healthy and together with the scions taken from them were discarded. The remaining plants, both in the pots (numbering 64 and in addition 27 not used) and in the hop garden (5 plants used and named above), have shown no signs of mosaic disease. The plants in pots were about a quarter of a mile distant from the stock plants used for grafting, which were all in the Experimental Hop Garden. Usually four scions were cut at a time and the greatest length of time from the taking of these to the insertion of the last was about an hour. Between each cut the razor or knife was dipped in absolute alcohol. The grafts were covered with lamp-glasses plugged with cotton wool and pressed well into the ground. Unless the soil was quite moist, it was found advisable to water the grafted plant at least once, and when the weather was dry to continue watering until the scion commenced to grow. In this process the water was kept as much as possible from touching the scion.

Between April 13th and June 3rd, 328 grafts were made; of these 216, or 65.9 per cent., were successful; the remaining 112 grafts either died or showed such weak growth that no results could be obtained from them. On June 9th the first appearance of mosaic disease on a scion was noticed.

In Table I the particulars are given of the grafting experiments in 1927. Section (*a*) contains a list of a number of different commercial varieties of hops (German, American, Danish and English) and also of Seedling and Male Hops, on all of which the scions developed mosaic disease. The symptoms appeared in all cases in unmistakable and characteristic form, evidenced by the curling and brittleness of the leaves, the mosaic mottling of the leaf tissue and cessation of growth of the stem, with the tip failing to climb and falling away from the string. Occasionally the tip of the stem wilted and died. Section (*b*) records the scions which remained healthy.

1928 (R. Williams). The method of grafting adopted was the same; in the earlier experiments the razor used in the preparation of scion and stock was kept in formalin (full strength) for some time previous to making the cuts and wiped with cotton wool before use. It was noticed afterwards that the blade still smelt strongly of formalin, but it was not until some hundred grafts had been made that it was realised that the traces of formalin affected the graft and caused the death of the scions. The method then adopted was as follows; two scalpels and two razors were kept immersed in absolute alcohol and were used alternately so that each one had been dipped in the alcohol for at least ten minutes before use. Fresh cotton wool was used each time to wipe the instruments dry. The grafting carried out from April 25th to the beginning of June, using this method of sterilisation, was successful, a fairly

high percentage of the grafts growing strongly although the cold wet spells of weather in the spring were unfavourable and slugs proved troublesome. The scions used were of the variety Eastwell Golding obtained from the farm of Mr Alfred Amos, at Wye. The "hills" from which the scions were taken were kept under observation and were free from mosaic disease throughout the season. A few grafts were also made with scions of the variety Tutsham and Rodmersham Golding. The Eastwell Golding scions were conveyed from Mr Amos' farm to the Experimental Hop Garden, two miles away, in a vasculum with moist cotton wool round the stems; an average of eight scions were taken at a time and only the freshest and most suitable were used, usually six. The lamp-glasses placed over the grafts were shaded by paper to ensure moist conditions.

Table I (a) gives particulars as regards those scions which developed mosaic disease, and Table I (b) as to those which remained healthy. As to the former, the symptoms of disease were the same as those obtained in the 1927 experiments. The upper leaves showed the characteristic mosaic mottling, curling and brittleness; the tip of the stem refused to climb and in some cases wilted and died back. In one case, OE 40 (ON 20), the scion reached the height of 274 cm., flowered and produced cones which showed distortion of bracts and bracteoles—one of the characteristic symptoms of mosaic disease. In addition, the leaves developed the symptoms.

### *Discussion of results.*

The experiments carried out indicate that varieties of hops cultivated in Germany, America, Denmark and England are "carriers" of the mosaic disease. This fact, which became apparent during the work in 1927, was so surprising that before its acceptance the confirmation of another year's investigation was thought to be necessary. An explanation of the results obtained in 1927 might have been forthcoming if the plants used for scions in 1927 had shown mosaic disease in 1928, but this was not the case. We will discuss the results obtained class by class. The varieties of hops grafted fall into the following groups: (1) German; (2) American; (3) Danish; (4) English; (5) Seedlings; (6) Male Hops.

#### *Group 1. German varieties.*

(a) Aischgründ, Hallertau, Württemberg, Spalt, Prackenfels, Beck'scher Frühhopfen, Lower Bavarian.

(b) Tettlinger, Hühhopfen.

Healthy scions of susceptible varieties (Tutsham, Eastwell Golding, Cobbs) when grafted on the German varieties included above under (a) all developed typical mosaic disease (see Table I). It would appear therefore that all these German varieties growing at Wye are "carriers."

All the varieties under (a), with the exception of Lower Bavarian, were originally obtained in 1911 from Prof. Wagner of Weihestephan, Germany, and were planted that year in the Experimental Hop Garden

at Wye. These varieties, comprising 25 plants, have during the period 1911-28 never shown any symptoms of mosaic, although this disease is very prevalent in the Experimental Hop Garden, where a considerable number of plants of other varieties in close proximity (in some cases actually adjoining) have, during the same period, been attacked and killed. The immunity shown is congruous with the now-ascertained fact of their being "carriers." Two other plants (clone-plants) of Hallertau used were obtained about 1912 from Dr Schmidt of Copenhagen under ref. no. Tysk B 149; the origin of these was likewise Weihestephän.

The Lower Bavarian variety was planted at Wye at some date before 1906 and its origin is unknown, but is probably Germany. The five "hills" which have been growing at Wye since 1906 have never shown symptoms of mosaic disease.

The mosaic disease has not apparently been reported from Germany<sup>1</sup>, but some German varieties are undoubtedly susceptible. Cases have occurred at Wye on the following: (1) plants obtained from some unknown source, prior to 1915, labelled "Upper Bavaria (Hallertau)"; the five plants were all destroyed by mosaic disease between 1917 and 1919; (2) plants obtained in 1916 from France (M. Blavier) of two varieties labelled "Alsace" and "Spalt." The entire stock, consisting of four plants of the former and five of the latter, has been destroyed by mosaic disease; (3) plants obtained in 1918 from Dr Quanjér of a variety labelled as a German variety and stated to have been obtained from Bavaria some 40 years previously and since cultivated at the Instituut voor Phytopathologie, Wageningen, Holland. Of this the stock of four plants has all been destroyed by mosaic disease during 1919-21.

No exact identification of the above four German varieties is possible; the fact, however, that they were wiped out by the mosaic disease while growing in close proximity to other German varieties, now shown to be "carriers," is of interest.

In view of the knowledge now gained that the Hallertau variety can serve as a "carrier," the following circumstances regarding an outbreak of mosaic disease seen some years ago on a certain farm in Kent, possibly acquire special significance. Between 1904 and 1908 a farmer obtained from Germany, through the medium of a London Hop merchant, cuttings of the Hallertau variety and grew them in a hop garden at Watlington, Kent. Some 200 sets were planted, with rows of the

<sup>1</sup> The disease called "mosaic Goldings" by Dr Blattny<sup>(1)</sup> does not agree, in the symptoms described, with mosaic disease as known in England.

mosaic-susceptible English variety Cobbs adjoining them on both sides. It was noticed in 1922, and for some years previously, that while the German variety remained healthy, many of the plants of Cobbs on both sides of them showed the mosaic disease, which was increasingly severe the nearer the plants were to the Hallertau variety. In 1922 the whole garden, including the Hallertau, was grubbed up and two years later was replanted with the Tutsham variety, since when no signs of mosaic disease have been noticed. The circumstantial evidence here would appear to be quite similar to that relative to the cases of the "carrier" seedlings M 45 and OD 8 (see pp. 378, 379).

In division (b), consisting of those stocks on which the susceptible scions remained healthy, are two varieties, Hühhopfen and Tettlinger. Both varieties were obtained in 1911 from Prof. Wagner; the first-named came from the Sortengarten des Hopfenbauzweigsvereines Hersbrucker Land in Hersbruck, and the second from the Hopfenvarietätengarten, Weißenstephan. The five plants of these varieties have occupied a similar position during 1911-28 to that of the varieties now proved to be "carriers," and have never shown any signs of mosaic disease. The evidence that the variety Hühhopfen is not a "carrier" appears strong. In 1927, 17 scions were taken from one plant of the susceptible variety Tutsham (R 1/50 a); 16 of these were grafted on to the varieties Prackenfels, Beck'scher Frühhopfen, Golden Cluster, L 36, M 45, OE 40 and OQ 17 and all developed mosaic disease, while the remaining one grafted on the variety Hühhopfen remained healthy. Similarly, in 1928, two scions of Rodmersham Golding (ref. nos. 33 and 26) which were grafted on another individual of Hühhopfen remained healthy.

In the case of the Tettlinger variety, one scion of the susceptible variety Rodmersham Golding grew to 215 cm. and remained healthy; the second scion, taken from a wild hop (ref. no. 84), grew to 275 cm. and remained healthy. The evidence that this wild hop was susceptible (and would have shown mosaic disease were the Tettlinger variety a "carrier") is strong, since six other scions taken from it, when grafted on to the "carriers" Grön, OF 24, O 39 and OQ 17, in every case developed mosaic disease.

Further work is now required to confirm these results and, if confirmation is obtained, to investigate whether the varieties Hühhopfen and Tettlinger are susceptible to mosaic disease or are immune varieties without being "carriers."

One puzzling feature occurred in connection with the variety Hallertau. While it appears clear from the results recorded in Table I

that the variety is a "carrier," since of two plants (ref. nos. R 1/24, R 2/12) one transmitted mosaic disease to two scions of Eastwell Golding and the other to two scions of Tutsham, another plant of Hallertau behaved differently. This plant (ref. no. R 2/3) was grafted with a scion of the wild hop (ref. no. 86) which grew to the top wire and remained healthy. Now this wild hop was used also in another experiment when a scion put on the "carrier" M 45 (see Table I) developed mosaic disease. The plant ref. no. R 1/24 was obtained from Prof. Wagner at Weihestephane, Germany, and the clone-plants ref. nos. R 2/3 and R 2/12, under the ref. no. "Tysk B 149," from Dr Schmidt, who informed us that the origin of these plants was Weihestephane. Further investigations are required here.

*Group 2. American varieties.*

Californian Golden Cluster, Oregon Golden Cluster, Humphrey, Red Vine Canada.

Healthy scions of the susceptible varieties Tutsham, Rodmersham Golding, Cobbs, Eastwell Golding, and of a wild hop, when grafted on all the above varieties, developed mosaic disease. In one experiment eight scions were taken from a plant (ref. no. R 1/82 a) of Rodmersham Golding; four were grafted on the Californian Golden Cluster, and all developed mosaic; the same was the case with one scion grafted on the "carrier" Seedling L 40, whereas two scions grafted on the variety Golding Grape and one scion on the susceptible variety Bates Brewer remained healthy. It would appear clear therefore that all these four American varieties are "carriers." The Californian, and Oregon, Golden Cluster and Humphrey show a healthy growth and normal appearance in every way, but the Red Vine Canada sometimes shows in its leaves symptoms of a virus disease. These symptoms were first noticed in a clone-plant (ref. no. R 1/74) obtained in 1914 from Dr W. W. Stockberger of the U.S. Dept. of Agriculture under ref. no. 18, the original cuttings being from J. J. Bennett, Waterville, New York. In 1927 it was observed that the leaves, both young and old, showed distinct mosaic mottling although this was semi-obscured by the dark green colour of the lamina; no other sign of disease was apparent, the leaves were not curled or brittle, the tips of the bine and laterals were not affected, and the growth and fertility were not impaired. The mosaic mottling was so different from that found in ordinary mosaic disease that the term "masked mosaic" is applicable. This appearance is perhaps seasonal and did not occur in 1928. It may easily have been passed over



in previous years. The other clone-plant used of this variety (ref. no. R 1/70) has not shown any abnormal coloration of its leaves. Five clone-plants have been grown at Wye.

The origins of the other American hops, now proved to be "carriers," are as follows. The Golden Cluster (ref. no. R 1/58) was obtained from Dr Stockberger in 1914 under the ref. no. 7 C, the original cuttings being from near Cosumne, California. Five clone-plants have been grown at Wye. The Californian Golden Cluster (ref. nos. E 24 (clone-plants R 3/55, OZ 93) and OZ 92 (clone-plant R 3/57)) was obtained by Mr Arthur Amos, in 1911, from Mr E. C. Horst, Perkins, near Sacramento, California. Six plants have been grown at Wye. The Oregon Cluster (ref. no. C 8) was sent as an "Oregon Male" by Messrs Wigan, Richardson and Co.'s agent in Oregon in 1908. Two clone-plants have been grown at Wye. The Humphrey (ref. no. R 1/67) was sent by Dr Stockberger in 1914 under the ref. no. 44, the original cuttings being from H. V. Pindar, Middleburg, New York. Four clone-plants have been grown at Wye.

None of the plants of the above varieties has shown mosaic symptoms.

Whilst the above female American varieties have proved to be "carriers" and resistant, all the male hops obtained from the United States have proved to be extremely susceptible to the mosaic disease, so much so that it has been almost impossible to maintain a stock at Wye. Male hops have been obtained from the following sources. (1) A male plant (ref. no. R 1/51) sent by Dr Stockberger in 1914 under the name of Golden Cluster, ref. no. 7 A. Six clone-plants were attacked and killed by mosaic in 1920-23. (2) An Oregon male (ref. no. F 9), sent in 1908 by Messrs Wigan, Richardson and Co.'s agent in Oregon, succumbed to mosaic disease in 1914, and a clone-plant in 1924. Another Oregon male (ref. no. F 6) of same origin was killed by mosaic disease in 1912, and a clone-plant in 1915. Another Oregon male (ref. no. F 7) of same origin died from the same cause in 1914, and a clone-plant in 1917. Three others (ref. nos. G 35, F 14, F 17) were killed in 1910, 1909 and 1914 respectively. (3) Rooted sets of male hops from California were obtained in 1914 from Messrs Horst Co., the cuttings being originally from the Russian River, Mendocino County, California. Eight plants (ref. nos. OY 1-OY 8) were attacked and killed by mosaic between 1918 and 1922.

### *Group 3. Danish variety.*

The Danish variety Grön was obtained from Dr J. Schmidt of Copenhagen about 1912, under the ref. nos. 199 and 198, the origin of

199 being given as Fyen, Denmark. Six clone-plants of 199 and four of 198 have been grown at Wye and have never shown mosaic symptoms. As will be seen from Table I, seven scions of Tutsham and of a wild hop were grafted on individuals of 199 and 198 and all developed mosaic disease. The variety Grön is therefore to be regarded as a "carrier."

Other Danish varieties sent by Dr Schmidt in 1911, it may be noted, are susceptible to mosaic disease. The variety Asperup (ref. no. 209) was attacked and killed by mosaic disease in 1915, and two clone-plants (ref. no. 210) perished of mosaic disease in 1915 and 1921. The variety Graa Rod from the Island of Fyen, Denmark, was killed by mosaic disease in 1921. A wild hop from Denmark (ref. no. Bork 291) proved to be very susceptible, nine clone-plants succumbing between 1919 and 1921.

*Group 4. English varieties.*

(a) Fuggles, Colgate, Allcorn, Golden Hop.

(b) Pye's Golding Grape.

The varieties in section (a) will be considered first. The Fuggles plant (ref. no. R 1/70 a) used in 1927 was of an English stock obtained from the late Mr G. Arnold. Two scions from a pot plant (ref. no. 47) of Tutsham were grafted on and both developed mosaic disease. One scion reached a height of 155 cm. and showed typical symptoms in the form of mottled, down-curved, and brittle leaves and, later, a non-climbing tip; the other scion grew to 73 cm. and, after showing mottled foliage, turned yellow and died.

In 1928 another plant (ref. no. R 1/68 a) of the same stock as above was grafted with Rodmersham Golding; the scion grew to 102 cm. and there were suspicious symptoms of disease, the leaves at many of the nodes showing a yellowish but not typical mosaic mottling, and the tip dying back in the manner characteristic of mosaic disease.

Other plants of the Fuggles variety were grafted in 1928. One plant (R 1/65) had been obtained in 1914 from Dr Stockberger, under ref. no. 9, from Sir A. Stephney's ranch, Agassiz, British Columbia, the original cuttings having come from East Kent, England. This plant was grafted with Rodmersham Golding; the scion grew to 48 cm. and a few of its leaves showed a faint but characteristic mosaic mottling.

Another plant (ref. no. R 4/97) is of an unknown (English) source, planted at Wye some time before 1917. The Tutsham scion grafted on reached to 43 cm. and its leaves showed faint mosaic mottling; the tip of the main stem and the laterals died back.

Table I.

*Grafting experiments with susceptible scions on various varieties of hops.**(a) Scions developing mosaic disease.*

1927 (D. MACKENZIE)

Stock		Scion		Suspected symptoms		Definite symptoms		Total height reached
Variety	Ref. no.	Variety	Ref. no.	Date	Height scion (cm.)	Date	Height scion (cm.)	by scion (cm.)
1. German varieties								
Aischgründ	R 1/12	Tutsham	38	5. vii.	23	3. viii.	23	23
"	R 1/12	Eastw. Gold.	76	—	—	9. vi.	61	62
"	R 1/20	"	72	3. viii.	278	30. viii.	325	325
"	R 1/20	"	78	9. viii.	295	30. viii.	317	317
Hallertau	R 1/24	"	63	5. vii.	33	12. vii.	33	33
"	R 1/24	"	62	22. vi.	35	29. vi.	40	41
"	R 2/12	Tutsham	1	19. vii.	80	23. viii.	128	128
"	R 2/12	"	1	23. viii.	195	5. ix.	195	195
Württemberg	R 1/32	Eastw. Gold.	71	22. vi.	110	3. viii.	200	215
"	R 1/32	"	66	—	—	3. viii.	310	310
Spalt	R 1/36	Cobbs	R 1/36 a	23. viii.	270	30. viii.	270	270
"	R 1/36	"	R 1/36 a	16. viii.	260	5. ix.	265	265
Prackenfels	R 1/39	Tutsham	R 1/50 a	—	—	16. vi.	85	100
"	R 1/39	"	R 1/50 a	—	—	16. vi.	85	123
Lower Bavarian	R 3/15	"	7	16. vi.	85	19. vii.	155	212
"	R 3/15	"	7	—	—	16. vi.	70	165
Beck'scher Frühhopfen	R 1/45	"	R 1/50 a	12. vii.	198	5. ix.	250	250
"	R 1/45	"	R 1/50 a	3. viii.	220	5. ix.	260	260
2. American varieties								
Golden Cluster	R 1/58	Wild Hop	83	12. vii.	40	9. viii.	30*	46
Red Vine Canada	R 1/70	Tutsham	31	26. vii.	31	9. viii.	31	31
"	R 1/74	"	46	—	—	16. vi.	72	195
Cal. Golden Cluster	R 24	"	R 1/50 a	22. vi.	35	29. vi.	38	112
"	R 24	"	R 1/50 a	16. vi.	47	22. vi.	47	47
"	R 3/55	"	R 1/50 a	12. vii.	187	19. vii.	190	210
"	R 3/55	"	R 1/50 a	12. vii.	112	19. vii.	120	150
"	OZ 93	Rodm. Gold.	R 1/82 a	5. vii.	141	12. vii.	155	335
"	OZ 93	"	R 1/82 a	16. vi.	51	22. vii.	60	143
"	OZ 92	"	R 1/82 a	—	—	16. vi.	40	46
"	OZ 92	"	R 1/82 a	16. vi.	75	22. vi.	81	115
"	R 3/57	Tutsham	R 1/50 a	—	—	16. vi.	45	49
Oregon Cluster	C 8	Cobbs	R 1/36 a	22. vi.	25	29. vi.	25	25
3. Danish variety								
Grön 199	R 2/2	Wild Hop	84	19. vii.	70	26. vii.	70	70
"	R 2/11	Tutsham	13	9. viii.	160	5. ix.	187	187
"	R 2/11	"	13	3. viii.	230	16. viii.	255	255
Grön 198	R 2/7	Wild Hop	85	26. vii.	195	16. viii.	260	265
"	R 2/7	"	85	26. vii.	95	9. viii.	125	135
"	R 2/16	Tutsham	17	9. viii.	155	16. viii.	158	158
"	R 2/16	"	17	9. viii.	290	16. viii.	310	315

\* Died back.

Table I (continued).

1927 (D. MACKENZIE) (continued)

Stock		Scion		Suspected symptoms		Definite symptoms		Total height reached
Variety	Ref. no.	Variety	Ref. no.	Date	Height scion (cm.)	Date	Height scion (cm.)	by scion (cm.)
<i>4. English varieties</i>								
Fuggles	R 1/70 a	Tutsham	47	22. vi.	90	29. vi.	95	155
"	R 1/70 a	"	47	—	—	16. vi.	56	73
Colgate	R 1/84	Rodm. Gold.	81 a	5. vii.	146	19. vii.	147	147
"	R 1/84	"	81 a	12. vii.	221	3. viii.	190*	221
"	R 1/85	"	81 a	12. vii.	164	26. vii.	165	165
"	R 1/85	"	81 a	12. vii.	165	19. vii.	165	165
Golden Hop	341	Wild Hop	81	29. vi.	33	12. vii.	33	33
"	288	Eastw. Gold.	63	—	—	16. vi.	51	65
"	(Bide's)	"	27	5. vii.	45	3. viii.	30*	45
"	(Bide's)	Tutsham	29	12. vii.	43	19. vii.	45	45
<i>5. New Seedling varieties</i>								
S 20	R 2/63	Tutsham	26	5. vii.	78	12. vii.	80	81
"	R 2/63	"	26	22. vi.	120	29. vi.	120	120
L 21	L 21	"	41	—	—	3. viii.	44	44
"	L 21	"	41	—	—	3. viii.	127	127
"	OZ 61	"	47	22. vi.	45	12. vii.	50	50
"	OZ 60	"	3	9. viii.	03	16. viii.	75	75
OP 21	R 4/49	"	31	—	—	16. vi.	60	130
"	R 4/49	"	31	22. vi.	88	29. vi.	91	122
"	OK 17	"	33	23. vi.	30	29. vi.	30	30
"	OK 17	"	34	23. vi.	59	29. vi.	60	61
"	R 4/43	"	8	22. vi.	58	29. vi.	60	85
"	R 4/43	"	8	16. vi.	54	22. vi.	60	125
"	R 4/50	Wild Hop	81	29. vi.	162	3. viii.	250	280
"	R 4/50	"	81	22. vi.	72	3. viii.	120	130
OF 30	OF 30	Eastw. Gold.	44	—	—	16. vi.	40	52
"	OF 30	"	44	16. vi.	47	23. vi.	49	49
"	R 2/49	Tutsham	13	—	—	16. vi.	52	53
M 54	OZ 17	"	22	—	—	3. viii.	210	Top†
"	OZ 17	"	22	26. vii.	210	3. viii.	Top	Top
"	221	"	36	—	—	16. vi.	50	157
"	221	"	36	22. vi.	81	29. vi.	92	208
"	222	Eastw. Gold.	68	16. vi.	70	22. vi.	78	185
"	222	"	62	16. vi.	60	22. vi.	66	147
"	158	"	73	16. vi.	80	22. vi.	99	260
"	158	"	79	22. vi.	90	29. vi.	107	280
L 36	L 36	Tutsham	16	26. vii.	65	3. viii.	65	65
"	L 36	"	16	19. vii.	74	3. viii.	80	80
"	J 51	"	R 1/50 a	5. vii.	107	19. vii.	125	150
"	J 51	"	R 1/50 a	5. vii.	109	19. vii.	145	185
L 40	40	Rodm. Gold.	R 1/83 a	—	—	3. viii.	78	78
"	40	"	R 1/82 a	—	—	16. vi.	63	64
M 45	163	Eastw. Gold.	72	29. vi.	131	12. vii.	160	160
"	G 15	Wild Hop	85	22. vi.	95	12. vii.	120	155
"	G 15	"	86	22. vi.	97	29. vi.	107	177
"	G 16	Tutsham	R 1/50 a	12. vii.	93	19. vii.	101	122
"	G 16	"	R 1/50 a	29. vi.	88	12. vii.	108	128
OF 24	11	"	40	22. vi.	58	29. vi.	61	67
"	V 42	"	4	22. vi.	72	29. vi.	72	72
"	V 42	"	6	22. vi.	94	29. vi.	97	98
"	OF 24	Wild Hop	84	16. vi.	49	23. vi.	50	50
"	OF 24	"	84	23. vi.	34	29. vi.	34	34
O 39	105	"	81	22. vi.	52	29. vi.	56	58
"	105	"	84	22. vi.	81	29. vi.	88	89
"	DD 30	Tutsham	42	12. vii.	43	26. vii.	45	45
"	DD 30	"	37	29. vi.	40	5. vii.	41	41

\* Died back.

† The height of the wirework varies from 12 to 14 feet, i.e. from 3·7 to 4·3 metres, from the ground.

Table I (continued).

1927 (D. MACKENZIE) (continued)

Stock		Scion		Suspected symptoms		Definite symptoms		Total height reached
Variety	Ref. no.	Variety	Ref. no.	Date	Height scion (cm.)	Date	Height scion (cm.)	by scion (cm.)
5. <i>New Seedling varieties (continued)</i>								
P 13	D 18	Cobbs	R 1/36 a	—	—	16. vi.	20	20
"	D 18	"	R 1/36 a	16. vi.	53	22. vi.	54	56
"	D 8	Tutsham	19	22. vi.	49	29. vi.	51	51
"	D 8	"	19	16. vi.	86	22. vi.	100	200
OH 8	OH 8	"	9	23. vi.	53	12. vii.	75	110
"	OH 8	"	32	—	—	3. viii.	25	25
B 7	B 7	"	19	3. viii.	125	30. viii.	140	140
"	B 7	"	0	12. vii.	102	19. vii.	120	175
"	E 7	Cobbs	R 1/36 a	9. viii.	50	16. viii.	55	55
OE 40	OE 40	Tutsham	29	29. vi.	102	5. vii.	112	147
"	X 38	"	1	29. vi.	29	26. vii.	29	29
"	X 38	"	1	23. vi.	109	29. vii.	114	135
"	ON 20	"	R 1/50 a	29. vi.	74	12. vii.	74	74
Y 86	Y 86	Cobbs	R 1/36 a	23. vi.	65	29. vi.	71	150
"	Y 86	"	R 1/36 a	—	—	16. vi.	71	160
"	Y 83	"	R 1/36 a	—	—	16. vi.	30	30
"	Y 83	"	R 1/36 a	—	—	16. vi.	33	35
OD 8	OD 8	Eastw. Gold.	70	29. vi.	32	26. vii.	40	40
"	OD 8	"	65	23. vi.	45	29. vi.	46	47
"	OG 59	Tutsham	26	—	—	9. viii.	25	25
"	EE 6	"	17	12. vii.	66	19. vii.	70	90
Q 52	OD 29	"	42	5. vii.	57	19. vii.	77	100
"	OD 29	"	42	5. vii.	125	12. vii.	150	260
"	OD 56	"	1	29. vi.	91	12. vii.	128	210
"	OD 56	"	1	23. vi.	120	5. vii.	155	260
OQ 17	OQ 17	"	R 1/50 a	—	—	3. viii.	98	100
"	OQ 17	"	R 1/50 a	—	—	3. viii.	88	88
"	OF 31	Eastw. Gold.	44	29. vi.	123	19. vii.	165	185
"	OF 35	"	52	5. vii.	62	26. vii.	83	92
"	OF 35	"	48	29. vi.	60	5. vii.	68	105
"	OF 36	Wild Hop	84	16. vi.	113	23. vi.	129	150
"	OF 36	"	84	23. vi.	120	29. vi.	131	200
OG 1	OG 1	Tutsham	56	23. vi.	29	29. vi.	29	30
310	OJ 22	"	20	29. vi.	27	3. viii.	27	27
"	OJ 22	"	20	12. vii.	56	19. vii.	56	56
"	OJ 12	Wild Hop	85	5. vii.	56	12. vii.	61	90
L 7	CC 1	Tutsham	31	5. vii.	108	12. vii.	108	108
"	CC 2	"	5	29. vi.	51	19. vii.	52	52

6. *Male Hops*

OI 22	OI 22	Tutsham	5	29. vi.	54	12. vii.	54	54
"	OI 22	"	5	—	—	12. vii.	60	70
G 27	II 2	"	43	16. vi.	79	23. vi.	81	81
"	II 2	Wild Hop	85	5. vii.	60	12. vii.	60	60
"	II 3	Tutsham	43	16. vi.	28	29. vi.	30	30
"	II 4	"	9	—	—	30. viii.	325	325
"	II 4	"	43	16. vi.	99	29. vi.	112	114
"	II 5	"	16	12. vii.	62	19. vii.	75	125
"	II 5	Eastw. Gold.	51	3. viii.	250	24. viii.	300	300
"	II 6	Tutsham	45	5. vii.	60	12. vii.	65	70
"	II 6	"	25	—	—	26. vii.	50	58
"	II 8	"	45	—	—	9. viii.	210	230
"	II 12	"	25	—	—	3. viii.	60	60
B 11.	T 36	"	5	12. vii.	78	26. vii.	82	82
"	T 36	"	6	29. vi.	43	12. vii.	55	56
"	T 43	"	9	22. vi.	79	29. vi.	81	84
"	T 54	"	30	5. vii.	30	3. viii.	30	30
"	T 66	"	30	19. vii.	36	3. viii.	36	36

Table I (continued).

1928 (R. WILLIAMS)

Stock		Scion		Total height of scion (cm.)	Symptoms
Variety	Ref. no.	Variety	Ref. no.		
1. <i>German varieties</i>					
Spalt	R 1/36	Eastw. Gold.	R 15/H 14	210	p.M.
Prackenfels	R 1/40	„	R 16/H 5	89	? M.
2. <i>American variety</i>					
Humphrey	R 1/67	Eastw. Gold.	R 1/H 7	74	M.
3. <i>English varieties</i>					
Fuggles (B.C.)	R 1/65	Rodm. Gold.	28	48	? M.
Fuggles	R 1/68 a	„	28	102	? M.
„	R 4/97	Tutsham	21	43	? M.
„	R 2/10	„	R 1/19 a	79	? M.
Golden Hop	288	Eastw. Gold.	R 3/H 5	117	? M.
Allcorn	OP 97	„	R 12/H 3	69	M.
4. <i>New Seedling varieties</i>					
S 20	S 20	Eastw. Gold.	R 1/H 17	59	? M.
L 21	OZ 59	„	R 1/H 22	74	M.
„	OZ 60	„	R 1/H 25	53	M.
OP 21	S 55	„	R 2/87	216	M.
„	S 60	„	R 2/H 8	206	M.
L 40	40	„	R 2/H 17	71	M.
458	457	„	R 3/H 2	125	M.
„	458	„	R 3/H 2	152	M.
OF 24	OF 24	„	—	125	M.
„	V 42	„	R 3/H 9	114	M.
B 7	E 7	„	R 4/H 3	175	M.
„	E 7	„	R 4/H 3	196	M.
OE 40	OE 40	„	R 4/H 5	109	M.
„	OE 40	„	R 4/H 5	69	M.
„	X 38	„	R 4/H 9	173	M.
„	X 38	„	R 4/H 6	64	M.
„	ON 20	„	R 4/H 7	274	M.
OD 8	EE 6	„	R 10/H 1	82	p.M.
„	OG 59	„	R 8/H 0	155	? M.
„	OG 59	„	R 8/H 1	41	? M.
„	OX 26	„	R 8/H 9	26	? M.
OQ 17	OF 35	„	R 10/H 14	152	M.
L 7	CC 2	„	R 12/H 1	114	? M.
5. <i>Male Hops</i>					
R 2/20	R 2/20	Eastw. Gold.	R 22/H 8	318	? M.
R 2/7 a	R 2/7 a	„	R 22/H 8	61	? M.

M. = Mosaic; p.M. = probable Mosaic.

Table I (*continued*).(b) *Scions remaining healthy.*

1927 (D. MACKENZIE)

Stock		Scion		Total height of scion (cm.)
Variety	Ref. no.	Variety	Ref. no.	
1. <i>German varieties</i>				
Tettnanger	R 1/15	Wild Hop	84	275
"	R 1/15	Eastw. Gold.	61	215
Hallertau	R 2/3	Wild Hop	86	Top
Hühbopfen	R 1/42	Tutsham	R 1/50 a	—
2. <i>English varieties</i>				
Pye's Gold. Grape	R 1/89	Tutsham	21	125
" "	R 1/91	"	10	283
" "	R 1/90	Rodm. Gold.	R 1/82 a	195
" "	R 1/90	"	R 1/82 a	300
" "	R 1/91	Eastw. Gold.	67	265
Bates Brewer	R 2/90	Rodm. Gold.	R 1/82 a	Top
"	R 2/90	"	R 1/83 a	Top
"	R 2/88	"	R 1/83 a	Top
3. <i>New Seedling varieties</i>				
OQ 17	OF 31	Rodm. Gold.	44	Top
4. <i>Male Hop</i>				
B 11	T 66	Tutsham	30	122

1928 (R. WILLIAMS)

1. <i>German variety</i>				
Hühbopfen	R 1/43	Rodm. Gold.	33	259
"	R 1/43	"	26	256
2. <i>English variety</i>				
Fuggles	R 4/99	Tutsham	R 1/23	64

The fourth plant (ref. no. R 2/10) had been obtained in 1911 from Dr Schmidt, under the ref. no. Eng. B 175, the original cuttings having come from Wye. The Tutsham scion grafted on grew to 79 cm. and its leaves showed very little and faint mosaic mottlings.

All the above experiments in 1928 must be regarded provisionally as doubtful cases of the transmission of mosaic disease<sup>1</sup>.

A number of plants of the Fuggles variety, growing in large pots, were grafted in 1928 by W. M. Ware. These plants were obtained from Mr Ingleby, Hereford, and were of three different stocks, *A*, *B* and *C*. The results are shown in Table II. The scions used were Eastwell

<sup>1</sup> One plant (ref. no. 4/99) of Fuggles, of an unknown English source, planted at Wye sometime before 1917, was grafted with a Tutsham scion; this grew to 64 cm. and remained healthy.

Table II.

*Susceptible scions (Eastwell Golding and Tutsham) grafted  
on the variety Fuggles.*

Order in which grafting carried out	Ref. no. of stock (variety Fuggles)	Ref. no. and variety* of scion	Date grafted	Height of scion on June 1st (cm.)	Scion		
					Healthy	Weak growth possibly due to virus	Un- doubted mosaic symptoms
23)	B 1	(E.G. W 46	27. iii.	30	—	×	—
11)		(T. 5	19. iii.	38	—	—	>
13)	B 2	(E.G. W 26	19. iii.	12.5	—	×	—
14)		(T. 24	19. iii.	79	×	—	—
16)	A 1	(E.G. W 32	27. iii.	37.5	—	—	>
15)		(T. 45	27. iii.	66.5	—	—	>
17)	A 2	(E.G. 70	27. iii.	165	×	—	—
18)		(T. 9	27. iii.	188	>	—	—
22)	B 3	T. 2	27. iii.	27	—	—	^
26)	B 4	(E.G. 70	2. iv.	14	—	×	—
27)		(T. 22	2. iv.	24	—	—	>
1)	C 1	(E.G. W 2	17. iii.	39	—	×	—
21)		(T. 30	27. iii.	116	×	—	—
10)	C 2	(E.G. W 20	19. iii.	16	—	>	—
2)		(T. 25	17. iii.	25	—	×	—
3)	C 3	(E.G. 76	17. iii.	17	—	×	—
25)		(T. 13	2. iv.	99	—	×	—
24)	C 4	(E.G. W 48	2. iv.	43	—	×	—
4)		(T. 0	17. iii.	111	×	—	—
5)	B 5	(E.G. 65	17. iii.	17	—	—	>
12)		(T. 22	19. iii.	71	—	—	×
6)	A 3	(E.G. 79	17. iii.	176	—	×	—
20)		(T. 23	27. iii.	62	—	—	>
7)	A 4	(E.G. 75	17. iii.	12	—	×	—
19)		(T. 12	27. iii.	135	—	—	—
8)	A 5	(E.G. W 16	19. iii.	17	—	×	—
9)		(T. W 18	19. iii.	18	—	×	—

\* E.G. = Eastwell Golding; T. = Tutsham.

Golding (E.G.) and Tutsham (T.). It will be seen that of the 27 scions grafted, 8, or 29.6 per cent., developed characteristic symptoms of mosaic disease. These were evident in the typical mottling of six of the 18 to 26 leaves on four of the scions and of eight out of 18 to 26 leaves on the other four.

Twelve scions, or 44.4 per cent., grew weakly and seven scions, or 25.9 per cent., remained perfectly healthy and reached heights of from 79 to 188 cm. The scions and stocks, when the grafting was carried out, were well matched and the operation was performed under the best conditions. Normally with such vigorous plants as stocks, the scions are expected to grow rapidly and to attain easily the height of 188 cm.



which in this experiment resulted only with the healthiest on one stock plant. All the Fuggles were of equal vigour and the poor growth of the twelve scions may perhaps be attributed to the same cause, *i.e.* virus attack, as in the eight which showed evident symptoms.

The stocks of Fuggles used were not clone-plants, but were obtained from a hop grower. Admixture is notoriously common in commercial stocks of varieties of hops, and the possibility must not be lost sight of that the plant A 2, on which both scions remained healthy and grew vigorously, may be of another variety, or a male hop. If this explanation may hold good for this case, we are still confronted with a difficulty as regards the plant A 3, where one scion (Eastwell Golding) remained healthy and grew to 176 cm., while the other (Tutsham) grew to only 62 cm. and developed mosaic disease. This case is somewhat similar to that of OQ 17 discussed on p. 376. The fact also may be noted that in the case of plant C 1, the Tutsham scion which remained healthy was taken from the same plant (ref. no. 30) as the one scion which remained healthy, in 1927, when grafted on the male hop B 11; two other scions from this same Tutsham plant developed mosaic disease when grafted, in 1927, on the same plant of B 11 and on another clone-plant.

Whilst it is clear that further investigations are here necessary, the positive results obtained in the three sets of experiments carried out in 1927 and 1928 appear to give safe grounds for stating that the Fuggles variety is, to some extent at least, a "carrier."

The Colgate plants grafted (ref. nos. R 1/84, R 1/85) were obtained from Mr A. W. Wallace, Battle, Sussex, in 1914. From that date to 1928, the four plants of this stock growing at Wye have never shown mosaic disease. Four scions of Rodmersham Golding were grafted on and all developed mosaic disease. It appears, therefore, that this variety is a "carrier."

In 1928 one plant of the variety Allcorn was grafted with one scion of Eastwell Golding. This scion, which grew to 69 cm., showed typical mosaic mottling on its leaves. Although the evidence here is slight, it would appear that the Allcorn variety, the origin of which is unknown, may be a "carrier." The variety was obtained in 1925 from the late Mr H. C. Wickham of Ticehurst.

The Golden Hop is a variety with yellow (golden) leaves. It is in use for horticultural purposes only. Its origin, so far as it can be traced, has already been dealt with(4). All of the five scions grafted (Eastwell Golding, Tutsham, and a wild hop) developed mosaic disease.

Section (b). The variety Pye's Golding Grape was obtained in 1914

from Mr William Pye, of Cuxton, Kent. No details of its origin are obtainable. Five scions of the varieties Tutsham, Eastwell Golding and Rodmersham Golding were grafted on three individuals (ref. nos. R 1/89-91) and all remained perfectly healthy. In the case of R 1/90 the evidence that the variety is not a "carrier" is particularly strong. Eight scions were taken from a plant of Rodmersham Golding (R 1/82 a); four were put on the California Golden Cluster, one on the Seedling L 40, two on the Golding Grape (ref. no. R 1/90) and one on Bates Brewer. The first two varieties named proved to be "carriers" and transmitted mosaic to the five scions; those on the Golding Grape and Bates Brewer remained healthy. The ten plants of Pye's Golding Grape grown at Wye since 1914 have never been attacked by mosaic disease. Investigations will now be carried out to ascertain whether this variety is susceptible or is truly immune without being a "carrier."

The variety Bates Brewer is known to be susceptible to the mosaic disease and it is therefore not surprising that it does not behave as a "carrier." The fact that the scions grafted on it remained healthy was useful evidence of the health of the parent plants.

#### *Group 5. Seedling varieties.*

All the Seedling varieties enumerated in Table I, with the exception of B 7, of which the origin is unknown, are Seedlings raised at Wye College; they have subsequently been sent as promising varieties to the Research Station, East Malling, for trial on commercial lines. The parentage of each and their cropping and other characteristics have been given in Annual Reports(5). The evidence both in 1927 and 1928 is conclusive that these Seedlings are "carriers." In many cases grafts were made on several clone-plants of the same Seedling; thus OE 40 was grafted with ten scions (of Tutsham and Eastwell Golding) and all developed mosaic disease; the same was the case with the nine scions grafted on OP 21. Scions taken from an individual plant of Tutsham remained healthy when grafted on the German variety Hühhopfen, but contracted mosaic disease when grafted on the Seedlings L 36, M 45, OE 40 and OQ 17. Similarly, scions taken from the wild hop (No. 84) remained healthy when grafted on the German variety Tettmanger, and developed mosaic disease on the Seedlings OF 24, O 39 and OQ 17. Scions taken from one plant of Rodmersham Golding remained healthy on the English variety Bates Brewer, and showed mosaic disease when grafted on the Seedling L 40; from another plant of Rodmersham Golding, the scions remained healthy on the variety Golding Grape and developed mosaic

disease on the Seedling L 40. Corroboration of the results obtained in 1927 was obtained in 1928; using a new stock of scions obtained from a commercial hop garden of the variety Eastwell Golding, it was found that mosaic disease was transmitted to every scion grafted on the Seedlings L 21, OP 21, L 40, OF 24, OE 40, OQ 17. In addition the Seedling 458 was found to be a "carrier." The symptoms shown were quite typical of the disease, the leaves of the stem, and also frequently of the lateral shoots, showing the characteristic mosaic mottling and becoming very brittle; the tips of the shoots ceased to climb, and in some cases died back. All these cases are classified as "Mosaic" in Table I, and those as "? Mosaic" in which the symptoms, though suggestive of the disease, in that the leaves showed traces of mottling and that sometimes the tip of the stem died back, were not marked enough to make the diagnosis certain. The rather weak growth made by many scions under the seasonal conditions was unfavourable for the manifestation of the disease.

The fact that every Seedling variety tested proved to be a "carrier" is at first sight certainly very surprising. The question naturally suggests itself as to whether there can be any correlation with parentage. Detailed information as to the parentage of each Seedling will be found in the Annual Reports<sup>(5)</sup>, but here a general summary may be given. Of the 22 Seedlings, 12 are of German and 4 of American parentage, while one was raised from the Golden Hop. In these cases the mother plant is either known to be or may be suspected of being a "carrier." Two Seedlings were raised from the Tolhurst variety, two from Canterbury Whitebine, and one from the Danish variety, Asperup—these three parents being all susceptible to mosaic disease. The male hop may here have had an influence.

The variety B 7 is of unknown origin and may possibly be a Seedling. It is of interest in being immune though showing the characters of certain English Golding varieties, all of which are susceptible to mosaic disease; it is now proved to be a "carrier."

It must be pointed out that only those Seedlings raised in the Nursery at Wye College which have shown over a number of seasons no signs of mosaic disease are sent to the Research Station at East Malling for commercial trial. All the Seedlings noted above have been thus selected and it is therefore not so surprising as it at first appears that all the twenty-two Seedlings tested have proved to be "carriers," since no certain case is known at present among hops of a variety being resistant to mosaic disease and not carrying the virus. Whilst being grown at

East Malling, none of the Seedlings has shown any trace of the disease, although commercial susceptible varieties growing adjacent have annually suffered severely (see Annual Reports<sup>(5)</sup>).

One of the most interesting anomalies was met with in 1927. Three clone-plants of the Seedling OQ 17 were grafted with six scions and all the scions developed mosaic disease. In 1928 one of these plants was again grafted and transmitted mosaic disease. It is clear therefore that OQ 17 is a "carrier." In 1927 a fourth clone-plant (ref. no. OF 3) of OQ 17 was grafted with two scions of Eastwell Golding taken from the same pot plant (ref. no. 44<sup>1</sup>); one developed mosaic disease while the other remained perfectly healthy, grew to over the top wire, flowered and produced cones. Certain other similar cases have been met with, viz. with Fuggles (see p. 373) and with the Male Hop ref. no. B 11 (see below).

Such cases can be explained hypothetically by assuming either (1) that the stock plant, although a "carrier" as regards certain shoots coming from the rootstock, produces other shoots which carry no virus or an insufficient amount; or (2) that the plant from which scions are taken may produce a bud-sport which is resistant to mosaic disease, whether carrying the virus or not.

#### *Group 6. Male Hops.*

Conclusive proof was obtained in 1927 that a male hop (ref. no. G 27) growing in the Experimental Hop Garden at Wye, and raised by A. Howard in 1904 from the Colgate variety, is a "carrier." Eleven scions of the varieties Tutsham and Eastwell Golding and of a wild hop all developed mosaic disease when grafted on seven clone-plants of G 27. With regard to another male plant (ref. no. B 11) of unknown origin, four scions of Tutsham grafted on three clone-plants (T 36, T 43, T 54) developed mosaic disease; on another clone-plant (T 66) two scions from the same plant (ref. no. 30) of Tutsham were grafted; one scion developed mosaic disease while the other grew to 122 cm. and remained healthy. The male hop B 11 is clearly a "carrier," and further investigations are necessary to explain why the one scion remained healthy. Clone-plants of both G 27 and B 11 have been grown at Wye and at the Research Station, East Malling, for several years past, and although almost certainly exposed to infection, have shown no trace of mosaic disease.

The male hop OI 22 (of the parentage Golden Hop × English Male

<sup>1</sup> Two scions of this plant grafted on the Seedling OF 30 both developed mosaic disease.

Hop × English Male Hop) was grafted with two scions of Tutsham and both developed mosaic disease (see Table I).

The existence of “carriers” among male hops<sup>1</sup> is a fact of considerable economic importance. Both G 27 and B 11 are of robust growth, of perfectly healthy appearance, have normal green foliage and are free-flowering, and on account of these characters have been propagated. Male hops are necessary in England for the production of a satisfactory crop, and are regularly planted by many farmers in the proportion of one male plant to 100–200 female plants. It is obvious that the planting of any “carrier” male hops will endanger the health of gardens which consist of commercial varieties susceptible to mosaic disease.

#### *General considerations.*

The discovery of the general prevalence in varieties of hops of the power of “carrying” the virus of mosaic disease without showing it raises many problems. One may speculate whether it will be found that the German, American and Danish varieties enumerated in Table I are “carriers” when growing in their own countries, or whether possibly they became “carriers” by infection abroad. With respect to the Seedlings enumerated, the indications are that these were “carriers” from the seed stage.

In the case of the English varieties, Fuggles and Colgate, the fact that these are grown in our country on heavy soils only, while the mosaic-susceptible Golding varieties require lighter soils, makes it unlikely that, in general, hop gardens of the latter varieties will be exposed to infection from the sources in question. On the Continent, where the Fuggles is now beginning to be cultivated on account of the resistance of its cones to the Downy Mildew, mosaic disease may well appear surreptitiously—assuming that all stocks of Fuggles are “carriers.”

The discovery of “carriers” among male hops makes it desirable that further investigations should be made to ascertain whether the male hops (which are of no definite variety) planted in commercial hop gardens belong to the class of “carriers.”

In the case of the New Seedling varieties, some of which have recently been used in brewing trials (8, 9) with promising results, it is obviously unsafe to plant such “carriers” in the proximity of susceptible Golding varieties; they must be grown either in separate gardens or near the

<sup>1</sup> In 1928 two male Seedling hops (ref. nos. R 2/20, R 2/7 a), both raised from the Golden Hop, were each grafted with one scion of Eastwell Golding. The leaves of both scions showed faint mosaic mottlings. These must be classed at present among the doubtful cases.

resistant ("carrier") commercial varieties Fuggles or Colgate. The question as to what distance should intervene between susceptible varieties and "carriers" can only be answered when it is known how mosaic disease is spread. At present nothing is known on this subject. Several ways in which the virus may be transmitted can be hypothecated. The Aphis, *Phorodon humuli*, and other insect parasites of the Hop may be agents; another possible animal vector may be the Eelworm, *Heterodera schachtii*, which Percival<sup>(3)</sup> and Duffield<sup>(2)</sup> have shown to infect the roots of the hop plant. The fine rootlets which develop in mass in the rich soil of the hop garden are torn apart and dispersed to some extent by the implements used in cultivating deeply, and fragments carrying eelworms may very possibly reach new uninfected gardens. The manual operation carried out each season of stripping off the leaves from the bines (stems) of the hop plants may play an important part in the spread of mosaic disease; the workers' hands, moistened with the sap of the leaves and shoots pulled off, may carry the virus and communicate it to adjacent varieties when the bines of these are in their turn wounded in the process of being stripped. In a similar manner it appears not improbable that in the annual operation of cutting the rootstock of the plant, the virus-infected sap may be transmitted by the knife from "carrier" varieties<sup>1</sup>. At the Research Station, East Malling, where all of the New Seedling Varieties mentioned above are being grown (together with "carrier" varieties such as Grön, Hallertau, Colgate and Fuggles) the present condition of affairs is such that as a row of plants of any susceptible Golding variety is planted in the proximity of the Seedling and other varieties, the row becomes almost at once decimated, and sometimes exterminated, by mosaic disease. Now that so many Seedling varieties have been proved to be "carriers," this rapid spread of mosaic disease could be explained on the assumption that in cultural operations, perhaps stripping, the Golding varieties become quickly infected.

A case where the Hallertau variety acted apparently as the "carrier," is noted on p. 362. Two further cases of the sudden appearance of the disease—inexplicable at the time but now probably to be explained by the agency of "carrier" varieties—may be mentioned. About ten years ago sets of the New Variety M 45 were distributed to two farmers at Watlington and Tunstall, Kent; they were planted for trial in gardens of mosaic-susceptible commercial varieties (Tutsham, Cobbs and Bramling),

<sup>1</sup> The possibility of soil infection in the case of certain mosaic diseases is indicated by the recent work of H. H. McKinney and R. W. Webb (*Journ. Agric. Research*, xxxv, 13 (1927) and xxxvi, 53 (1928)).

forming rows which were immediately surrounded by those of the susceptible variety. Under these conditions a severe outbreak of the disease occurred within three years of the planting<sup>1</sup>. Recently another New Variety, OD 8, has been tried on the same farm at Wateringbury and again the mosaic disease appeared in the adjoining rows in the hop garden. Our present knowledge that both M 45 and OD 8 are "carriers" provides us with the probable explanation in these cases, although we are still ignorant of the means whereby in nature the virus is transported from "carrier" plants. The transmission of mosaic disease has been demonstrated only by grafting, but it is abundantly clear that the disease spreads by some other means in hop gardens.

It may be pointed out here that the mosaic disease of the hop is comparatively new to this country. It was first described in 1923, but records kept at Wye show that the first occurrences noted there were during the years 1907 to 1910. It is idle, in our present ignorance, to speculate as to the origin of the disease, whether it is specific to the hop, or whether it may possibly be communicated from some other plant. If German and American varieties have always been "carriers," this source has been present in Kent for many years past. A hop garden of German varieties existed at Wye College some time before 1900. In other parts of Kent, and also in Sussex, the German variety Hallertau has been grown on an experimental scale by a few hop growers; American varieties (Palmer's Seedling and Oregon Cluster) were planted experimentally by hop growers at Horsmonden and Teynham, Kent, many years before 1900.

## II. RESISTANCE OF THE FUGGLES VARIETY WHEN GRAFTED ON A "CARRIER."

In the experiments made by D. Mackenzie in 1927 and tabulated below, scions of the variety Fuggles were grafted on varieties now known to be "carriers."

In every case the scion grew well and remained healthy<sup>2</sup>.

<sup>1</sup> Further details will be found in the Sixth Annual Report<sup>(5)</sup>.

<sup>2</sup> In other experiments carried out by D. Mackenzie in 1927, and by R. Williams in 1928, the variety Fuggles was grafted with scions taken from another individual of the same variety (not clone-plants). Eleven experiments of this nature were carried out. All the scions grew well, in many cases reaching the top wire, remained healthy, and produced cones. In two experiments the variety Fuggles (ref. nos. R 4/99, R 1/61 a) was self-grafted with the same result. In 1927, two scions of Fuggles were grafted on the German variety Schwetzingen (R 1/48); both reached the top wire and remained healthy. It is not known yet whether this German variety is a "carrier."

Table III.

*Scions of the variety Fuggles grafted on "carrier" stocks.*

Stock		Scion (Fuggles)	
Name of variety	Ref. no.	Ref. no.	Total height reached (cm.)
Golden Cluster	R 1/57	R 1/62 <i>a</i>	Top*
" "	R 1/57	R 1/62 <i>a</i>	375
" "	R 1/58	R 1/62 <i>a</i>	300
Humphrey	R 1/66	R 1/63 <i>a</i>	Top
"	R 1/66	R 1/63 <i>a</i>	380
"	R 1/67	R 1/64 <i>a</i>	165
"	R 1/67	R 1/64 <i>a</i>	260
Red Vine Canada	R 1/70	R 1/64 <i>a</i>	Top
L 21	OZ 59	R 1/61 <i>a</i>	Top
"	OZ 59	R 1/61 <i>a</i>	Top
OP 21	S 55	R 1/63 <i>a</i>	Top
"	S 55	R 1/63 <i>a</i>	340
"	S 60	R 1/63 <i>a</i>	Top
"	S 60	R 1/63 <i>a</i>	Top
L 40	CC 10	R 1/61 <i>a</i>	310
"	CC 10	R 1/61 <i>a</i>	Top
458	458	R 1/62 <i>a</i>	Top
"	458	R 1/62 <i>a</i>	Top
"	457	R 1/62 <i>a</i>	Top
P 13	D 13	R 1/61 <i>a</i>	Top
"	D 13	R 1/61 <i>a</i>	Top
OH 8	A 14	R 1/62 <i>a</i>	Top
"	A 14	R 1/62 <i>a</i>	Top
"	OW 20	R 1/62 <i>a</i>	145
Y 86	GG 13	R 1/61 <i>a</i>	Top
"	GG 13	R 1/61 <i>a</i>	Top

\* The top of the wirework varies between 12 and 14 feet, i.e. between 3·7 and 4·3 metres, from the ground.

It has already been shown (11) that scions of the variety Fuggles when grafted on a hop plant severely affected by mosaic disease can grow vigorously, remain healthy, and produce hops which ripen their seeds normally. It has now been shown that similarly the Fuggles variety is not infected when grafted on "carriers."

#### SUMMARY.

1. When scions of varieties susceptible to mosaic disease were grafted on German, American, Danish, English and Seedling varieties, they developed typical mosaic disease. It appears, therefore, that "carrier" varieties are common amongst commercial varieties of hops. Certain male hops also proved to be "carriers."

2. In a few cases susceptible scions grafted on "carrier" stocks remained healthy. These anomalous cases require further investigation.



3. As a rule, the "carrier" varieties show no trace whatever of mosaic disease; the leaves are of normal colour and the plant shows normal growth with high fertility.

4. A case is recorded where one "carrier" variety (Red Vine Canada) exhibited in one season nascent symptoms of disease. The term "masked mosaic" is suggested for the appearance shown.

5. Scions of the commercial variety Fuggles, when grafted on "carrier" plants, invariably remained healthy.

We take this opportunity of thanking Mr Alfred Amos, Spring Grove Farm, Wye, for his kindness in giving us every facility for obtaining scions from his hop garden.

#### REFERENCES.

- (1) BLATTNY, C. (1927). Peronospora (Falscher Meltau) des Hopfens. *Trav. Instit. rech. agronom. Rép. Tchécoslov.* xxvii a, 249.
- (2) DUFFIELD, C. A. W. (1925). Nettlehead in Hops. *Ann. App. Biol.* xii, 536.
- (3) PERCIVAL, J. (1895). The Eelworm Disease of Hops. *Journ. S.E. Agric. College*, i, 5.
- (4) SALMON, E. S. (1920). On Forms of the Hop (*Humulus Lupulus* L. and *H. Americanus* Nutt.) resistant to Mildew (*Sphaerotheca Humuli* (DC.) Burr.). *Ann. App. Biol.* vi, 293.
- (5) — (1922-28). *Fifth to Eleventh Reports on the Trial of New Varieties of Hops, 1921-27, East Malling Research Station.*
- (6) — (1923). The "Mosaic" disease of the Hop. *Journ. Min. Agric.* xxix, 927.
- (7) SALMON, E. S. and WARE, W. M. (1925). Virus diseases and the Grafting of the Hop. *Gard. Chron.* LXXVII (3rd ser.), 320.
- (8) SALMON, E. S. (1927). Notes on Three New Varieties of Hops. *Journ. Inst. Brewing*, xxxiii, 12.
- (9) — (1927). Notes on the Ten New Seedling Varieties of Hops used in the Brewing Experiments at Manchester, 1926. *Ibid.* xxxiii, 570.
- (10) SALMON, E. S. and WARE, W. M. (1928). Inter-Specific Grafting in *Humulus*. *Gard. Chron.* LXXXIII (3rd ser.), 396.
- (11) — (1928). The Mosaic Disease of the Hop; Grafting Experiments, I. *Ann. App. Biol.* xv, 342.
- (12) THRUPP, T. C. (1927). The Transmission of "Mosaic" disease in Hops by means of Grafting. *Ibid.* xiv, 175.

(Received November 27th, 1928.)

## STUDIES ON POTATO VIRUS DISEASES

VI. FURTHER EXPERIMENTS WITH THE VIRUS OF A  
POTATO MOSAIC UPON THE TOBACCO PLANT

BY KENNETH M. SMITH, D.Sc., Ph.D. (CANTAB.).  
(*Potato Virus Research Station, School of Agriculture, Cambridge.*)

(With Plates XIV–XVII and 1 Text-figure.)

## CONTENTS.

	PAGE
1. Introduction . . . . .	382
2. Production of an increase in virulence of the virus by progressive needle inoculation of tobacco ringspot . . . . .	383
3. Comparison of needle and aphid ( <i>M. persicae</i> ) inoculations of healthy potato from a tobacco plant affected with the virulent type of ringspot . . . . .	385
4. Comparison of needle and aphid ( <i>M. persicae</i> ) inoculations of healthy tobacco from tobacco plants affected with ringspot in both its ring-like and its virulent form . . . . .	386
5. Analysis of a comparative series of aphid ( <i>M. persicae</i> ) and needle inoculations with potato mosaic and tobacco ringspot . . . . .	388
6. Response of different varieties of tobacco to needle inoculation with the virulent virus . . . . .	391
7. Non-transmission of ringspot through the seed . . . . .	392
8. Distribution of the virulent virus in an affected tobacco plant . . . . .	392
9. Attenuation of the virulent virus by an apparent inherent resistance in individual tobacco plants . . . . .	393
10. Some preliminary attempts to develop in tobacco plants an immunity to the virulent ringspot virus . . . . .	394
11. Behaviour of the progeny of potato plants inoculated with tobacco ringspot the preceding year . . . . .	395
12. Discussion . . . . .	396
13. Summary . . . . .	397
Explanation of Plates XIV–XVIII . . . . .	399

## 1. INTRODUCTION.

In a previous communication in this series<sup>1</sup>, a description was given of some preliminary experiments with the virus of potato mosaic upon the tobacco and of the resulting ringspot disease in that plant. Experi-

<sup>1</sup> Smith, Kenneth M. (1929). Studies on Potato Virus Diseases. IV. *Ann. App. Biol.* xvi, No. 1.

mentation has been continued, and in the present paper some further facts concerning the behaviour of tobacco ringspot under varying conditions are presented. In the very large numbers of different inoculations described in this paper, where inoculation by both needle and aphid has become a routine, the writer has not considered it necessary to give details of numbers of plants used, temperatures, incubation period of the virus, etc.

Acknowledgment is due to Mr F. T. Brooks and Dr D. Keilin with whom the writer has discussed some of this work.

## 2. PRODUCTION OF AN INCREASE IN VIRULENCE OF THE VIRUS BY PROGRESSIVE NEEDLE INOCULATION OF TOBACCO RINGSPOT.

It has been shown already<sup>1</sup> that progressive needle inoculation of one strain of tobacco ringspot through successive tobacco plants resulted in a greatly increased virulence. Experiments have been conducted with four separate strains of ringspot, primarily induced as before by needle inoculation of tobacco with mosaic potato, var. Arran Victory, two in Virginia and two in White Burley. These viruses were passed through a series of tobacco plants by means of needle inoculation, and in every case a marked increase in virulence resulted. This virulence was such as to cause the death of very young tobacco plants in every case, and to produce a very severe necrosis in older seedlings. The behaviour of older seedlings so inoculated was fairly constant, the necrotic disease killed all the plant except the young central shoot. After a period of two to three weeks, the plant commenced to grow again (Plate XIV, fig. 1) and new leaves were formed, upon which a severe and sometimes brilliant mosaic-like mottling developed. Increase in virulence becomes apparent at the 4th or 5th successive inoculation. In the case of Virginia, at the 3rd or 4th series large numbers of double, more rarely treble, concentric rings appear in clusters around and along the needle scratches (Plate XIV, fig. 2). As the inoculations progress, the rings lose their clear-cut concentric form and merge through necrotic spots into large lesions (Plate XIV, fig. 3), until a point is reached where the degree of virulence is such that, especially in White Burley, the leaves die so rapidly after the appearance of the first symptoms, that neither rings nor lesions have time to develop before the whole leaf collapses and dies. The first symptom in the development of the most virulent type of disease, that is at the stage when actual rings are no longer formed, is fairly constant. It takes

<sup>1</sup> Smith, Kenneth M. *Loc. cit.*

the form of a very marked "clearing" of the veins, in which the veins of the younger leaves become bright yellow and necrotic and stand out in a strongly defined network. Following upon this the veins of the older leaves except the inoculated ones also stand out. Clear window-like patches appear in the younger leaves which rapidly grow limp, shrivel and disappear. Within a few days of the appearance of first symptoms, all the outer leaves are dead and the central shoot alone is left alive, and in some cases this also is killed. As a rule however in older plants the central shoot persists, and later commences to grow again. Apart from the formation of actual rings "clearing" of the veins is a most constant and characteristic first symptom, and it is an invariable first symptom of the aphid-produced disease when so transmitted from ring-spot tobacco to healthy tobacco. It is often the case with young plants that the *inoculated* leaves remain green and apparently living until the very end, long after the younger leaves have disappeared. The only symptom which appears to develop upon the inoculated leaves is the double concentric ring, and that usually when the inoculations have reached the 3rd or 4th plant. Plate XVII, fig. 1 shows the type of lesion which develops in White Burley tobacco. During these progressive needle inoculations therefore, the virus exhibits a fairly constant sequence of symptoms. Starting with the double rings produced by direct inoculation from mosaic potato, as inoculation progresses these rings become more pronounced and necrotic, gradually losing their sharp outline and joining together into irregular groups. Later comes the production of the intensified clearing of the veins, followed by clear window-like lesions, the whole leaf hanging limply without turgidity. It is at this stage of the progressive inoculations that the plant dies down and remains stationary for some weeks. Growth then recommences and what is virtually a new plant is formed. On the new leaves thus formed, the symptoms enter upon another phase; both rings and necrotic lesions have usually disappeared and their place is taken by mottling of a most pronounced type, which is occasionally sufficiently brilliant to simulate ordinary tobacco mosaic (Plate XVII, fig. 2). There may develop also white, yellow, or dark green patches, while occasionally very heavy lines of dark green may appear along the veins. What may be the significance of this sequence and variety of symptoms is not at present known. It might be suggested that the virus itself is undergoing some transformation, and these successive symptoms represent transitional stages in such a transformation, or they may represent merely a process of adjustment between the plant host and the accentuated virus,

whereby the plant is finally enabled to continue its growth, although the virus is still present in the sap.

3. COMPARISON OF NEEDLE AND APHIS (*M. PERSICAE*) INOCULATIONS OF HEALTHY POTATO FROM A TOBACCO PLANT AFFECTED WITH THE VIRULENT TYPE OF RINGSPOT.

Needle inoculation of healthy potato plants with the ordinary form of tobacco ringspot produces a mosaic in which symptoms are intensified and infective power greatly increased. Similar inoculation into potato with the virulent form of tobacco ringspot, that is, ringspot passed through a number of succeeding tobacco plants, produces an additional factor. On the young leaves of a potato plant so inoculated appear first the symptoms of the intensified mosaic, characteristic of ordinary ringspot inoculation, later however the lower leaves of the potato plant commence to die and death proceeds rapidly up the plant, the leaves first turning yellow and then shrivelling completely. The whole plant may finally die (Plate XV, figs. 1, 2). Death of the whole plant however does not always take place, often the lower leaves only die; just as in the case of tobacco affected with the virulent virus death may be confined to the older leaves of the plant. A series of needle inoculations was made from a potato plant, which was dying from the effects of the virulent ringspot virus, back into healthy White Burley tobacco seedlings. These developed the typical virulent form of the disease and rapidly died, a parallel series of inoculations from another potato similarly inoculated in another experiment produced the same effect on the Connecticut variety of tobacco. This seems to show that passage through healthy potato does not involve any marked change in the virulent virus. Aphides have been induced to carry infection with regularity to healthy potato from tobacco affected with the virulent ringspot. The disease resulting in the potato was symptomatically the same as that produced by aphid infection from ordinary ringspot tobacco, *i.e.* an intensified form of mosaic. When this disease was returned to healthy tobacco seedlings by needle, the dark green lines typical of aphid infection were produced. Comparison of the respective inoculations of healthy potato by aphid and needle from tobacco affected with the virulent virus shows that the lethal character of the disease is not transmitted by the aphid.

4. COMPARISON OF NEEDLE AND APHIS (*M. PERSICAE*) INOCULATIONS OF HEALTHY TOBACCO FROM TOBACCO PLANTS AFFECTED WITH RINGSPOT IN BOTH ITS RING-LIKE AND ITS VIRULENT FORM.

It has been shown<sup>1</sup> that needle and aphid (*M. persicae*) inoculations respectively from a mosaic potato into tobacco or from ringspot tobacco into healthy tobacco produce two diseases symptomatically different. Further experiments on this subject have been performed using as sources of infection ringspot tobacco plants exhibiting the disease in both ring-like and virulent forms. As regards aphid transmission of ordinary ringspot, a very large series of experiments has been performed. Aphid transmission has been carried out on six varieties of tobacco, *i.e.* Virginia, White Burley, Connecticut, Havana, Maryland and Kentucky. These six varieties were used in the experiments both as healthy plants to be infected and as sources of infection. All six varieties developed the characteristic clearing of the veins as a first symptom on the young leaves, followed by the appearance of the typical dark green lines or spots, mostly along the veins, which is the invariable appearance of the aphid-induced disease from a ringspot source (Plate XV, fig. 3). It was also found possible to pass the aphid-induced disease through potato and back to tobacco by the needle where it still retained its original character (Plate XV, fig. 4). This seems fairly conclusive evidence that the aphid-induced disease is not merely a varietal reaction on the part of one particular kind of tobacco. The variety Virginia is very resistant to aphid infection but can be inoculated by means of the needle with the aphid-induced disease from another variety. Further experiments were performed using a tobacco plant affected with the virulent virus as the source of infection. Needle inoculation from this plant produced a similar though slightly more severe disease in the inoculated plants, while aphid inoculation from the same source produced the characteristic green lines. This experiment is illustrated in Plate XVI, figs. 1, 2, 3. That this aphid-induced disease is a definite virus of an infectious nature and constant character has been proved experimentally. Once induced in tobacco by the aphid (*M. persicae*) it is possible to transmit it to other tobacco plants by means of both needle and aphid, and the symptoms remain constant in the inoculated plants. This disease has been needle-inoculated from a tobacco plant in which it was originally induced by the aphid, through eight succeeding tobacco plants (Plate XV, fig. 3), the symptoms remaining constant throughout the series, *i.e.* clearing of the veins followed by the development of the typical mottling and lines of

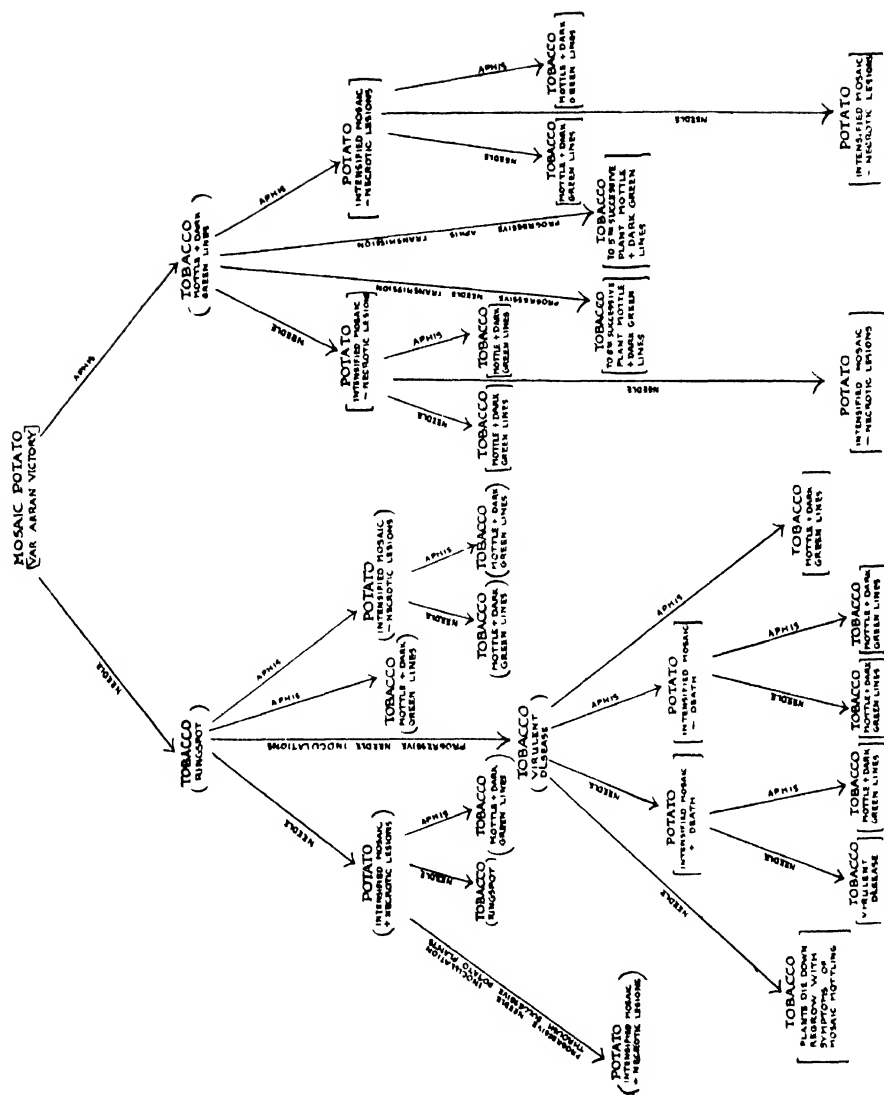
<sup>1</sup> Smith, Kenneth M. *Loc. cit.*

darker green. Little, if any, increase in the intensity of the symptoms was apparent. A parallel series of progressive inoculations was carried out using the aphid instead of the needle, and was carried to the 5th series of plants; the same result was apparent here except that the disease showed a tendency to attenuation in the last series. There are one or two possible suggestions for this production of two apparently distinct virus diseases from a single source of infection. It cannot be that the aphid picks up one constituent part only of a possible virus complex, because it has been shown already that *M. persicae* can transmit potato mosaic to healthy potatoes unchanged. It may be that the difference in symptoms is due to the different mode of inoculation. If this were the case, however, it should hardly be possible to pass on the aphid-induced disease unchanged by means of the needle. In this connection an attempt was made to simulate aphid inoculation of tobacco by pricking the leaves with a fine needle, lightly dipped in the virus, about a dozen fine punctures being made in the leaves of each plant. A series of White Burley plants was inoculated in this manner, using a strain of virulent virus. The disease produced, however, was of the ordinary severe type and not that characteristic of aphid infection. Another explanation may be that some substance in the sap of the tobacco plant reacts upon the saliva of the aphid and consequently upon the virus presumably contained therein. It is quite possible that in reality the actual difference between the aphid-induced disease and the various forms of tobacco ringspot is not so great as would appear from their symptoms, and further experiment may indicate the liaison existing between them. On one or two occasions a mottling disease very similar in appearance to the aphid-transmitted disease has resulted from needle inoculation from mosaic potato, notably some examples of mosaic in the variety President. This however is an exceptional occurrence. The most common result of needle inoculation of tobacco from mosaic potatoes of one or more varieties is the formation of the typical double rings or necrotic spots. Using the same tobacco plant affected with the virulent form of ringspot as source of infection it has been found possible to infect healthy tobacco seedlings with two separate diseases. Firstly, by inoculating the seedlings with the aphid the disease showing the characteristic dark green lines was produced, and then needle inoculation with the juice from the same source produced the virulent type of disease. The presence of the first or aphid-induced disease does not prevent the rapid development of the second or needle-induced disease.

#### 5. ANALYSIS OF A COMPARATIVE SERIES OF APHIS (*M. PERSICAE*) AND NEEDLE INOCULATIONS WITH POTATO MOSAIC AND TOBACCO RINGSPOT.

In a previous communication a description was given of respective aphid and needle inoculations from mosaic potato to tobacco plants. It has been shown that such needle inoculations produced ringspot while inoculations with the aphid from the same source produced a mottling accompanied by lines and spots of darker green. In Text-fig. 1 an attempt has been made to portray diagrammatically the large numbers of needle and aphid inoculations which have been carried out with potato mosaic and tobacco ringspot and to show the resulting symptoms in each case. Considering the left half of the diagram first, it will be seen that beginning with mosaic potato, var. Arran Victory, needle inoculation from that source produces ringspot in tobacco. If that is passed by needle into potato an intensified form of the original mosaic is produced together with numbers of small necrotic lesions. Aphid transmission from the same ringspot to healthy potato produces an intensified form of mosaic very similar in appearance to that produced by the needle but lacking the necrotic lesions. Taking the needle-produced intensified mosaic in potato as the source of infection, needle inoculation of that into tobacco reproduces tobacco ringspot while aphid inoculation from the same source into tobacco produces the typical mottle accompanied by lines of darker green. Considering now the aphid-induced intensified mosaic in potato as the source of infection, needle and aphid inoculations from that into healthy tobacco both produce the same disease, *i.e.* the mottle accompanied by dark green lines. Next, reverting to the original ringspot tobacco, progressive needle inoculation of this virus through successive tobacco plants produces the virulent type of disease; incidentally it may here be pointed out that aphid inoculation from ordinary ringspot tobacco produces the typical mottle and dark green lines. Taking now as source of infection a tobacco plant affected with the virulent form of ringspot, further needle inoculation of this into tobacco kills all the outer leaves of the plant leaving the central shoot only alive, which after a week or so starts into new growth producing leaves upon which different symptoms of a mosaic type develop. Needle inoculation into healthy potato with the virulent form of ringspot produces first of all the intensified mosaic followed usually by rapid death of the plant. Needle inoculation from this plant back into tobacco reproduces the virulent ringspot; aphid inoculation from the same source produces the typical dark green lines disease. Considering now aphid transmission





from the tobacco affected with the virulent form of ringspot; first into potato, a similar intensified mosaic is produced as by needle but lacking its lethal character. Aphis and needle transmission from this last source into tobacco produces in each case the typical dark green lines in the inoculated plant. Aphis inoculation of the virulent form of ringspot into healthy tobacco produces the same dark green lines as are produced by aphis inoculation from the ordinary form of ringspot into healthy tobacco. That concludes the left-hand half of the diagram where the inoculations had a common source of infection in needle-induced ringspot of tobacco. In the right-hand half of the diagram the common source of infection is a tobacco plant with the mottle and dark green lines disease induced by aphis transmission from mosaic potato. Needle and aphis inoculations respectively from this tobacco plant into healthy potato produced the same intensified mosaic in each case. This disease was symptomatically identical with the disease produced by aphis inoculation of potato from ordinary ringspot tobacco, and differed apparently from that produced by needle inoculation from ringspot tobacco in that the small necrotic lesions were absent. Reverting to the intensified mosaic produced in potato by both needle and aphis from the aphis-infected tobacco, needle and aphis inoculations of both these diseases back into tobacco produced in all four series the same mottle and dark green lines as were exhibited by the tobacco which was the original source of infection. In the centre of the right-hand half of the diagram are represented progressive needle and aphis inoculations respectively from the same source of infection through succeeding tobacco plants.

The progressive needle inoculations were carried up to the 8th plant (Plate XV, fig. 3) and the progressive aphis inoculations up to the 5th plant. Little, if any, increase in virulence was noted in either case, possibly the symptoms were somewhat brighter towards the end of the needle sequence, while a tendency to attenuation was apparent in the case of the aphis. In reviewing the inoculations set forth in Text-fig. 1, it will be seen that most of the possible cross inoculations have been carried out. Perhaps the outstanding fact arising from these comparative inoculations is the constant character of the aphis-induced disease; wherever an aphis transmission enters into a series, inoculation into tobacco subsequent to that, either by aphis or needle, produces the mottle and dark green lines disease. The respective symptoms arising from the two methods of transmission most nearly approach a common point of similarity in the potato plant. The text-figure shows that transmission to potato from tobacco either by needle or aphis produces the intensified

form of mosaic whether the source of infection in the tobacco be induced by needle or aphid. The apparent difference between the needle- and aphid-induced mosaics in potato lies in the production of small necrotic lesions when the disease is needle-transmitted from ringspot tobacco, and also in the subsequent death of the potato plant (Plate XV, figs. 1, 2) when the needle transmission is effected from a tobacco with the virulent disease. It should be noted however that in the case of needle and aphid infections of healthy potato from tobacco affected either with ringspot or the "aphid disease," the intense mosaics produced are really very similar, and if, as sometimes happens, the small necrotic lesions are absent, then symptomatically the diseases are identical. Yet in spite of this close resemblance, the text-figure shows that they do not behave similarly when returned to tobacco. In all the inoculations so far carried out, a *needle*-produced mosaic in potato from ringspot tobacco when returned to tobacco by needle reproduced ringspot or spot-necrosis, while an *aphid*-produced mosaic in potato either from a ringspot tobacco or an aphid-infected tobacco when returned to tobacco by aphid or needle produced the typical dark green lines.

It would appear therefore that the aphid does not transmit some element of the virus which is concerned with the production of the more necrotic and lethal symptoms.

#### 6. RESPONSE OF DIFFERENT VARIETIES OF TOBACCO TO NEEDLE INOCULATION WITH THE VIRULENT VIRUS.

Further experiments have emphasised the greater resistance of Virginia tobacco over White Burley. In a series of needle inoculations of the virulent virus made into twelve White Burley and twelve Virginia plants, all small seedlings of the same age and size, the White Burley plants all died, but half the Virginia plants partially recovered and survived for some months albeit severely infected. Again, it has been found many times that a strain of virus, which on Virginia still produces double rings, produces severe necrotic lesions when inoculated into White Burley. The age of the plant at the time of inoculation also governs to some extent the degree of severity of the resulting disease. Very small seedlings of White Burley when inoculated with the virulent form of the virus invariably die, but half-grown plants, on the other hand, often survive and may exhibit a less severe form of the disease. Four other varieties of tobacco were tested, Kentucky, Maryland, Havana and Connecticut, and showed a susceptibility more comparable to that of White Burley. They were all less resistant than Virginia.

In the case of needle inoculation into Virginia with a strain of virulent virus, it often happens that the plant manages to grow away from the disease to a certain extent. Such a plant may live for months and even flower and set seed. It exhibits the typical severe lesions on the lower leaves, but the upper part of the plant appears normal, the young leaves being green, symptomless and apparently healthy. A series of parallel inoculations was made into healthy tobacco seedlings from these green leaves, and into other seedlings from the older leaves which showed lesions. All the inoculated plants developed the disease to the same degree of virulence, thereby showing that the virus was still active in the green apparently normal leaves. Although the degree of virulence of the virus was equal in both green and diseased leaves, it does not always follow that this virulence is necessarily as great as at the time of original inoculation into the plant.

#### 7. NON-TRANSMISSION OF RINGSPOT THROUGH THE SEED.

Seed was saved from twelve tobacco plants of the two varieties, Virginia and White Burley, affected with ringspot, including the virulent form and the aphid-induced disease. In all about 500 seedlings were grown; all proved healthy. This experiment seems to show that ringspot is not commonly, if at all, transmitted by the seed, and thereby it falls into line with other virus diseases of plants. Subsequent inoculation of these plants with ringspot produced the disease, there being no difference in their reaction from that exhibited by seedlings from healthy plants.

#### 8. DISTRIBUTION OF THE VIRULENT VIRUS IN AN AFFECTED TOBACCO PLANT.

The leaves of six White Burley seedlings were needle-inoculated with an inoculum made from the roots of a tobacco plant affected with the virulent virus. In ten days all six plants developed the normal virulent type of disease, thus showing that the virus is present in the roots of affected plants. The converse of this experiment was also performed, the roots of six healthy White Burley plants were inoculated with an inoculum made from a leaf of a tobacco plant affected with the virulent virus. In 14 days these six plants developed the virulent type of disease.

#### 9. ATTENUATION OF THE VIRULENT VIRUS BY AN APPARENT INHERENT RESISTANCE IN CERTAIN INDIVIDUAL PLANTS.

Large numbers of needle inoculations into tobacco plants have shown that an average of one plant in six, even a smaller average of the susceptible variety White Burley, possesses what is presumably an inherent resistance to the virulent ringspot virus. Such plants, instead of developing the disease in its severe form, show weaker symptoms which often exhibit a tendency to return to ring formation. Experiments were made in order to determine whether this phenomenon was due to a definite attenuation of the virus by some inherent character in such individual plants, or whether the plant was in some way more tolerant of the virus and so was acting as a partial "carrier." A series of needle inoculations was made from such a plant (Virginia) into healthy Virginia and White Burley seedlings. The Virginia plants developed a mild type of disease very similar to that exhibited by the source of infection, the White Burleys developed a disease more severe than in the Virginias, but much less severe than was produced in similar White Burley plants by the same strain of virus which had not been passed through the Virginia. That the White Burleys would be more seriously affected than the Virginias was to be expected in the light of the greater susceptibility of the former. This experiment seems to suggest that the virus is definitely attenuated by its passage through plants possessing this apparent inherent resistance. That this inherent resistance is easily broken down is shown in the succeeding section. This phenomenon should be distinguished from that described in section 6 where a plant has developed the virulent form of the disease and then has partly recovered. In such a plant, although the young leaves show no symptoms, the virus seems to retain much of its original intensity. In reviewing the above experiments the possibility must be considered that this attenuation of the virus may be due, not to an inherent resistance in individual plants, but to the reception by such plants of minute quantities only of the virus, by accident of inoculation, and that such minute quantities of the virus are capable of causing an attenuated, though constant, form of the disease.

10. SOME PRELIMINARY ATTEMPTS TO DEVELOP IN TOBACCO  
PLANTS AN IMMUNITY TO THE VIRULENT VIRUS.

For this work the susceptible variety White Burley was used, and the first experiment was as follows: six White Burley plants were needle-inoculated with a strain of the virulent virus which had been killed by heating over a spirit lamp. After 21 days, no symptoms having developed, the plants, together with three other White Burleys to serve as controls, were inoculated with the same strain of virus unheated. In 11 days all the plants developed the ordinary virulent form of the disease, there being no apparent difference between the plants treated with the "dead" virus and the control plants. In the next experiment a number of tobacco plants were selected which had already been inoculated with the most virulent form of the virus but owing to some inherent resistance, the symptoms developed had not been of the severe necrotic character usually exhibited by this virus but had appeared in a modified form of ring-like tendency, severe enough in some cases but not sufficiently so to interfere with the growth of the plants. Twelve plants in all were selected for this experiment, and they were needle-inoculated a second time with the same strain of virus. Half the plants were given the virus from the same sources from which they had already received it, and half from affected plants later in the sequence of successive inoculations; these latter would therefore receive a virus possibly slightly more virulent in character. In ten days all twelve plants developed the virulent form of disease, the typical yellowing of the veins appearing on the youngest leaves and later on the older ones. Clear patches then developed on the leaves which lost all turgidity and hung limply like thin cloth. Of these plants only the central shoot was left alive; later this commenced new growth, the new leaves exhibiting a marked mosaic-like, though still necrotic, type of mottling. It is thus apparent that, under certain conditions, it is possible to inoculate a tobacco plant twice with the same virus, if there exists some factor of resistance to the first inoculation. In addition the experiment shows that the inherent resistance is easily overcome, and also that the presence of the virus in an attenuated form in the plant is no bar to the rapid development of the virulent form of the virus and confers no immunity to later infection. In a further experiment an attempt was made to ascertain the result of double inoculation of tobacco plants, where the first inoculation consisted of the virulent virus artificially attenuated by alcohol. The first series consisted of six

White Burley seedlings inoculated with a suspension of the virus in 75 per cent. alcohol, the leaf from the source of infection being ground up with the alcohol and inoculated immediately into the plants. The typical virulent disease developed in 14 days and killed the seedlings. The experiment was then repeated keeping the virus in 75 per cent. alcohol for varying periods until the symptoms produced in the inoculated plants were very faint, showing that the virus had been attenuated by the alcohol. Subsequent inoculation of these plants with virus free from alcohol produced the typical virulent disease, thus showing that no immunity had been conferred by the attenuated virus. The last experiment in this series deals with the double inoculation of a tobacco plant from the same source of virulent ringspot but using the aphid as the first method of inoculation. Six plants of White Burley, affected with the typical dark green lines disease induced by means of aphid from a plant with virulent ringspot, were needle-inoculated from the same source. The disease developed normally, the symptoms of both diseases being present together in the same plant. These attempts to produce an immunity in tobacco are only of a preliminary nature, but so far as they go they offer little hope that such an immunity can be experimentally conferred.

#### 11. BEHAVIOUR OF THE PROGENY OF POTATO PLANTS INOCULATED WITH TOBACCO RINGSPOT THE PRECEDING YEAR.

Numbers of tubers from potato plants (vars. Arran Victory and President) needle-inoculated in 1928 with tobacco ringspot were planted in 1929. The resulting plants showed the same intense mosaic, accompanied by small necrotic spots, as was exhibited by the parent plants the preceding year. Needle inoculation from these plants into Virginia tobacco produced typical ringspot. Into White Burley spot necrosis was produced in some cases and, in others, double rings along the inoculation scratches. In this last respect the symptoms differed from those produced by direct inoculation into White Burley with ordinary potato mosaic, as double rings do not usually form along the scratches on a first inoculation from ordinary potato mosaic to tobacco. Occasionally, also, juice from potato plants arising from parents affected with the intensified form of mosaic produced severe necrosis in White Burley tobacco plants. Juice from these potato plants was also inoculated into healthy Arran Victory, symptoms of the intensified mosaic developing in nine days, thereby showing that the increased infective power was still retained.

These facts seem to indicate that the change in the virus of potato mosaic induced by its passage through tobacco may be of a permanent nature.

## 12. DISCUSSION.

By improvements in methods of technique the writer has induced the aphid, *M. persicae*, to carry infection with fair regularity from infected tobacco to healthy potato. As is shown by Text-fig. 1, it has been found possible to infect potato (vars. Arran Victory and President) with the intensified mosaic by *M. persicae* from tobacco affected with ringspot, the spot necrosis form, the aphid-induced disease, and the virulent form induced by progressive inoculation. All these diseases thus aphid-transmitted to potato have produced identical symptoms, and the mosaics produced have also behaved similarly when returned to tobacco by needle or aphid. The chief points in the behaviour of tobacco ringspot when produced by needle inoculation from mosaic potato, and of the mottle and dark green lines disease produced by aphid inoculation also from mosaic potato, may be briefly compared. Firstly ringspot can be greatly increased in virulence and infective power by progressive needle inoculation through successive tobacco plants. When needle-inoculated into potato in its ringspot form, a mosaic is produced with enhanced symptoms and increased infective power; when inoculated into potato in its virulent form the same mottling is produced, but is usually followed by the death of the whole or part of the potato plant. When these forms are returned to tobacco by the needle, they produce substantially the same disease as was shown before passage through potato. Comparing, then, this behaviour with that of the disease produced by aphid from mosaic potato, progressive inoculation through succeeding tobacco plants either by aphid or needle does not produce increase in virulence, at least when taken as far as the 8th successive plant. Return of this disease to healthy potatoes, either by needle or aphid, produces apparently the same intensified mosaic as was produced by needle inoculation with the ringspot form but lacking the necrotic lesions usually present with the latter. When returned to tobacco by aphid or needle the same disease is produced as was present before passage through potato.

It will be seen then that both the needle- and aphid-produced diseases are fairly constant in their behaviour. The change of symptoms, whatever it may portend, which is produced by the aphid is apparently permanent, as shown by the behaviour of the disease when subsequently passed on



by the needle through either potato or tobacco. On the other hand, the close similarity between the symptoms produced in potato by the two diseases seems to indicate that the difference between them is in reality only slight. It is difficult at the present stage of the work to do more than state the facts as they are, trusting to later research to throw light on the true meaning of these apparent transformations of the virus.

### 13. SUMMARY.

Further experiments with the virus of a potato mosaic upon the tobacco plant are described.

(1) Marked increase in virulence has been shown to occur in ringspot by progressive needle inoculation through succeeding tobacco plants. Four strains of ringspot arising from four distinct mosaic potato plants (var. Arran Victory) have been so experimented with in White Burley and Virginia tobaccos.

(2) Comparison of needle and aphid (*M. persicae*) inoculations of healthy potato plants from a tobacco plant affected with the virulent ringspot shows that although both methods of inoculation produce an intensified mosaic in the potato plant, that produced by the needle is often fatal to the potato.

(3) Comparison of needle and aphid (*M. persicae*) inoculations of healthy tobacco plants from a tobacco plant affected with the virulent ringspot shows that while the needle reproduces the virulent disease, the aphid produces a mottle and dark green lines disease, apparently the same as that produced by aphid transmission from the ordinary type of ringspot.

(4) An analysis is given of a large comparative series of aphid and needle inoculations with potato mosaic and tobacco ringspot. This indicates that the aphid-induced disease is an infectious virus of constant character. The experiments also show that the aphid fails to transmit the more lethal characters of the disease.

(5) The response of different varieties of tobacco to the virulent virus was examined. Six varieties were used in the experiments: White Burley, Virginia, Kentucky, Maryland, Havana, Connecticut; Virginia was found to be the most resistant, and White Burley the most susceptible, while the other four varieties approximated to White Burley in susceptibility.

(6) Large numbers of seeds were sown which had been derived from tobacco plants affected with ringspot and its various modifications. In no case was the disease transmitted through the seed.

(7) Experiment showed that the virulent virus was present in the roots of an affected tobacco plant; and also that needle inoculation of the roots of healthy tobacco plants with the virulent virus produced the typical disease.

(8) It was found that certain individual tobacco plants, both Virginia and White Burley, seem to possess an inherent resistance to the virulent virus which attenuates it. Needle inoculation of such a plant with the virulent virus produces faint symptoms only. When juice from this plant is passed on to other tobacco plants the virus still shows weak symptoms, as compared with those produced by the same strain of virus before its passage through such a resistant plant. This seems to indicate that certain plant individuals have the power of attenuating the virus.

(9) A description is given of some preliminary attempts to produce experimentally in tobacco plants an immunity to the virulent virus. Plants were first inoculated with a suspension of "dead" virus, killed by heating, and three weeks later they were inoculated with the same strain of virus unheated. Experiments were also performed giving, first, inoculations with the virus artificially attenuated, and then inoculating with the virulent virus. Tobacco plants affected with the aphid-produced disease were also needle-inoculated with the virulent virus. A series of resistant individual plants which had developed only a mild disease after inoculation with the virulent virus, was inoculated a second time, with the same strain of virus in some cases and in others with a different strain. In all the above experiments the normal virulent disease developed, no immunity having been conferred.

(10) Tubers, from potato plants infected the previous year with tobacco ringspot, were grown in order to study the behaviour of the virus after a winter spent in the resting tuber. Plants were produced exhibiting all the symptoms of the intensified mosaic characteristic of infection with tobacco ringspot which were exhibited by the parent plants the year before. This seems to indicate that the change induced in the virus of a potato mosaic by passage through tobacco may be of a permanent nature.



Fig. 1



Fig. 2.



Fig. 3.

SMITH. —STUDIES ON POTATO VIRUS DISEASES (pp. 382-399).





Fig. 1.



Fig. 2.



Fig. 3.



Fig. 4.





Fig. 1



Fig. 2.

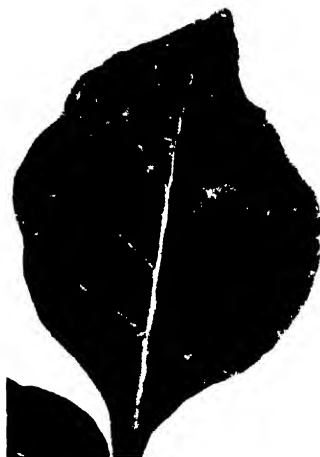


Fig. 3.





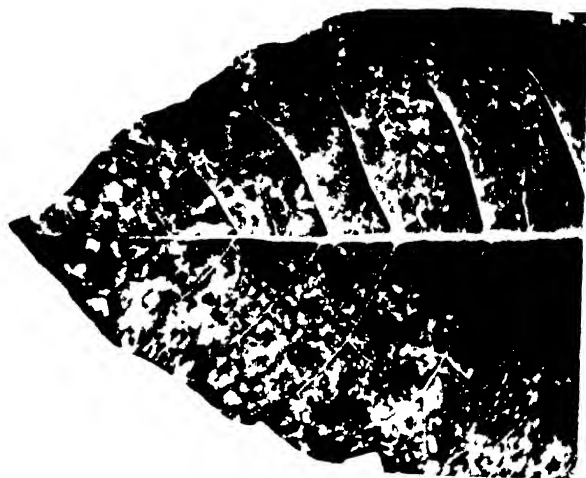


Fig. 2.

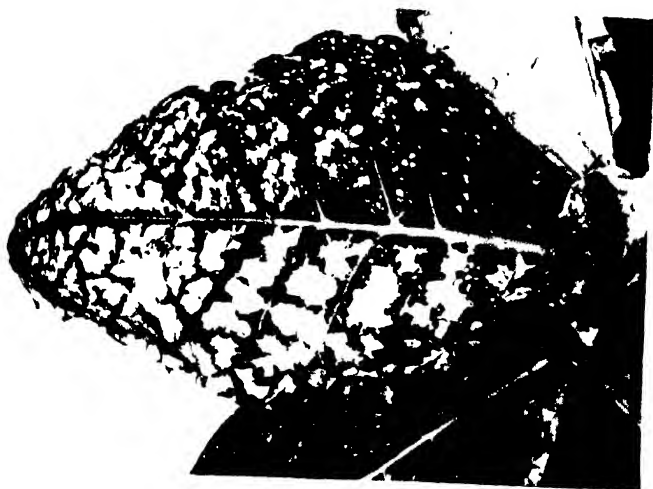


Fig. 1.

SMITH. SCUDILS ON POTATO VIRUS DISEASES (pp. 382-399).



## EXPLANATION OF PLATES XIV—XVII

## PLATE XIV.

- Fig. 1. White Burley tobacco plant starting into new growth, after inoculation with the virulent form of ringspot. Note the old leaves killed by the virus.
- Fig. 2. Rings forming along the needle inoculation scratches on the leaf of tobacco, var. Connecticut, fourth successive inoculation.
- Fig. 3. Leaf of Virginia tobacco plant, showing the severe lesions produced by the virulent virus. Length of leaf 12 cm.

## PLATE XV.

- Fig. 1. Potato plant, var. Arran Victory, needle-inoculated with the virulent virus from tobacco, showing death of the lower leaves.
- Fig. 2. Potato plant, var. Arran Victory, killed by needle inoculation with the virulent virus from tobacco.
- Fig. 3. Leaf of White Burley tobacco showing the typical appearance of the aphid-induced disease. This particular plant was the eighth in a series of successive needle inoculations of the disease, after it had once been produced in tobacco by the aphid. No appreciable increase in virulence was obtained.
- Fig. 4. Leaf of White Burley tobacco affected with the aphid-induced disease. In this case the disease, after production in tobacco by the aphid, had been needle-inoculated through potato and back to tobacco. The symptoms remained unchanged.

## PLATE XVI.

The three figures in this plate show comparative needle and aphid inoculations into tobacco from the same source of infection.

- Fig. 1. The source of infection, a White Burley tobacco plant affected with the virulent virus.
- Fig. 2. A White Burley tobacco plant which has been needle-inoculated from the plant shown in Fig. 1.
- Fig. 3. Leaf of a White Burley seedling aphid-inoculated from the plant shown in Fig. 1. The development of the dark green lines is plainly shown.

## PLATE XVII.

- Fig. 1. Leaf of White Burley tobacco showing the large lesions produced by the virulent virus.
- Fig. 2. Leaf of White Burley tobacco plant showing the mosaic-like mottling sometimes produced by the virulent virus, after the plant has partially recovered from the disease.

Photographs by C. W. Williamson.

*(Received March 4th, 1929.)*

*DALDINIA CONCENTRICA* ATTACKING THE  
WOOD OF *FRAXINUS EXCELSIOR*

By THÉRÈSE E. PANISSET, A.R.C.S., B.Sc.  
(Imperial College of Science and Technology, London.)

(With 23 Text-figures.)

AN English timber merchant, on cutting up a butt of an ash tree, found that the wood was beautifully marked with irregular, brown to black, bands, lines and patches, yet the wood was sufficiently firm to permit of its being made into ornamental boxes. Such wood is so far from being unfamiliar to timber merchants that, according to Mr J. Richardson, it has the name of "calico-wood."

EXTERNAL APPEARANCE OF "CALICO-WOOD."

The wood was streaked with irregular, wavy, purplish-brown to black stripes, which were not confined to any one zone of the annual rings but



Fig. 1. "Calico-wood" cut longitudinally, showing the dark streaks and the blackened pore zone.

ran along and across them; these lines were usually 1 to 5 mm. in width, but in some longitudinal or transverse sections they appeared as circular patches or rings. The large spring vessels of every annual ring had black contents (Fig. 1).

#### ANATOMY OF NORMAL ASH WOOD.

Before describing the microscopical structure of this discoloured wood, a description of normal ash wood will be given.

A normal annual ring of *Fraxinus excelsior* is made up of: (1) on the inside a zone of spring wood, the pore zone, consisting of wide vessels surrounded by wood parenchyma, between which lie fibres with slightly thickened walls; (2) a wide middle zone with thicker-walled fibres, and scattered smaller vessels surrounded by wood parenchyma; (3) on the outside a limiting layer consisting of several rows of wood parenchyma with scanty, small, very thick-walled vessels. The spring vessels are very large compared with the size of the other elements; their walls bear numerous bordered pits, the canals of which are elongated owing to the wall-thickening, and widen suddenly into the cavity; the canals may be branched once or twice. The summer vessels are similar, becoming smaller and more thick-walled towards the outer part of the annual ring. The wood parenchyma is always associated with the vessels, surrounding the small ones on the inner and outer sides of the large ones. In the late summer wood, however, the parenchyma spreads away between the vessels in the form of an arc in transverse section. The wood fibres have simple pits and form the main groundwork of the wood; they are lined with a thin layer of protoplasm. There are also "intermediate fibres" which are transitional in structure between the wood parenchyma and the fibres; and their position is commonly near the true parenchyma. The medullary rays are numerous, 1 to 3 cells wide, 5 to 20 cells deep, the ray being lenticular in shape in tangential view; the inter-cellular spaces in the ray are well marked in tangential sections.

As regards the contents of the lumina, in my specimen all the cells of the wood parenchyma and of the medullary rays had colourless, yellow, or light brown, globular contents of an unknown composition, sometimes together with starch grains. No fats, tannins, or mucilages were found stored in the cells to any extent, but starch was contained in quantity and De Bary<sup>(1)</sup> states that in a stem of *Fraxinus* when 40 years old an abundance of starch was found by Gris in all the annual rings.

## ANATOMY OF INFECTED WOOD.

For microscopical examination the wood was cut into small blocks which were submerged in acetone and de-aerated, and then left in a solution of 12 gm. cellulose acetate in 100 c.c. acetone until soft enough (10–14 days) for cutting by microtome. The stains used on the sections were lactic blue, safranin and picro-aniline blue, and gentian violet.

In the wood, before artificially staining it, large patches of the section were brown, due to the change in colour of the wood walls and their contents. These patches had no defined limits, but included an indefinite number of cells, the colour being darkest in the centre of the patch and gradually fading out at the edges. In the walls, the pits contained a brown substance, and sometimes the middle lamella was the most deeply coloured, particularly near the pits and along the intercellular spaces, the rest of the wall taking on a lighter shade of brown. In transverse sections the brown colour extended farthest along the medullary rays, from a discoloured patch to the colourless wood. The cell-contents of the parenchyma were stained a deep brown (Figs. 2–5).

Fungal hyphae, present in large quantities, were of three kinds:

(1) Very fine, segmented, *colourless* hyphae with granular contents were seen when the sections were stained with lactic blue or picro-aniline blue. Branching was sparse and irregular, the hyphae as a rule were not matted together, but passed from cell to cell, through all the tissues, by means of the pits in the cell-walls. These hyphae were present in the brown and the colourless wood and occurred in the vessels, fibres, medullary rays and parenchyma cells.

(2) *Brown to black*, segmented, hyphae were visible in unstained sections. These were much wider than the colourless hyphae, and were of two kinds:

(a) In the large spring vessels these hyphae were *dichotomously branched*, short and brittle; the tips of the branches were pointed and at the junction of the branches the hypha was slightly swollen; there were no contents. This type of mycelium occurred in wood having brown or colourless walls, most abundantly in the spring vessels but also in the summer vessels, fibres and parenchyma cells. Discoloration of the ash wood was consequently due to the presence of this black mycelium as well as to the darkening of the walls.

(b) The other kind of brown hyphae were sparsely *branched in an irregular way*, wider than the dichotomous hyphae, and found almost exclusively in the medullary ray cells of the brown-coloured wood.

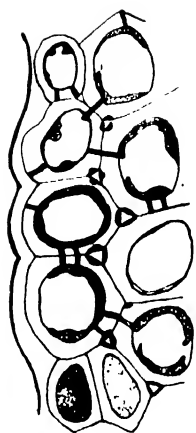


Fig. 2.

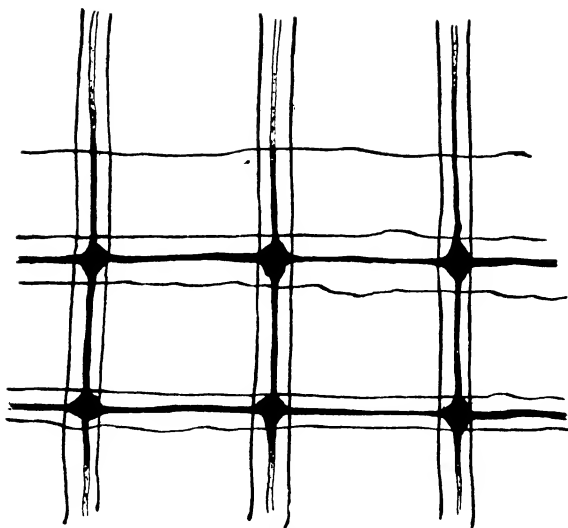


Fig. 4.

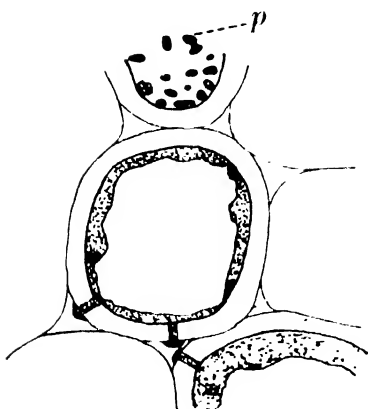


Fig. 3.

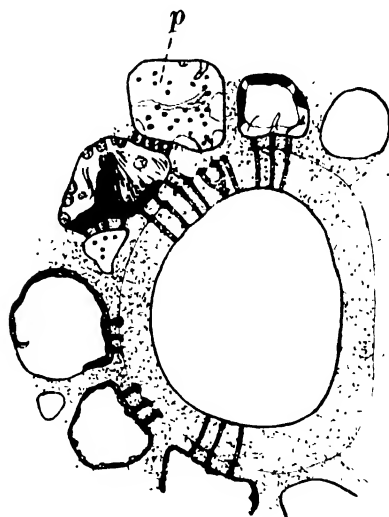


Fig. 5.

Fig. 2. Tangential longitudinal section of part of medullary ray, showing brown staining in pits.

Fig. 3. Part of Fig. 2 enlarged. *p* = pits in surface view.

Fig. 4. Radial longitudinal section, showing brown stain in middle lamellae of fibres and medullary ray cells.

Fig. 5. Transverse section of a small vessel and parenchyma, showing stain deepest in pits and paler in rest of wall. *p* = pits in surface view.



Fig. 6.

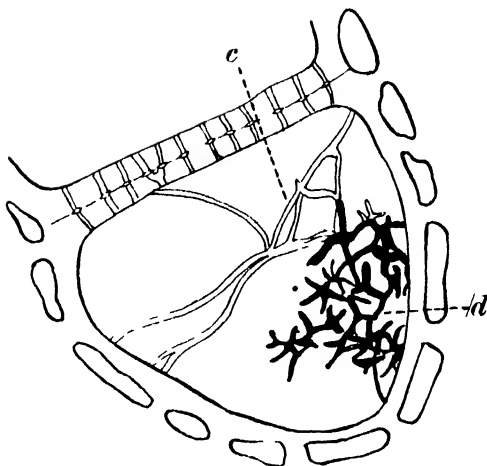


Fig. 7.

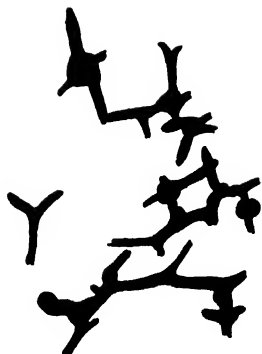


Fig. 8.

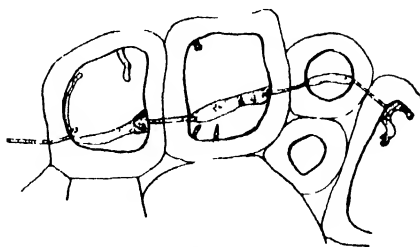


Fig. 9.

Fig. 6. Radial longitudinal section of medullary ray, showing straight brown hyphae in cells and passing through the pits.

Fig. 7. Transverse section of a vessel and parenchyma, showing black dichotomous hyphae (d) and colourless hyphae (c) in vessel.

Fig. 8. Black dichotomous hyphae from a vessel, enlarged.

Fig. 9. Transverse section, showing colourless hypha passing across the fibres via the pits.



Peg-like branches from these hyphae passed through the pits of the medullary ray cells, and the hyphae were empty (Figs. 6-9).

The general appearance of the contents of the parenchyma cells was not changed much by the presence of the fungus or of the brown colour, except that they were stained by the latter. The globular bodies described as occurring in the cells of normal ash were present in the brown and the colourless wood. Starch grains were scanty, but a few were seen in brown patches as well as in the colourless wood.

Lignin, pectin and cellulose occur in the walls of normal ash. The wood infected by this fungus remained firm, and the cell-walls did not show any change in thickness or structure except where the pits were perforated by the hyphae. Phloroglucin with hydrochloric acid was

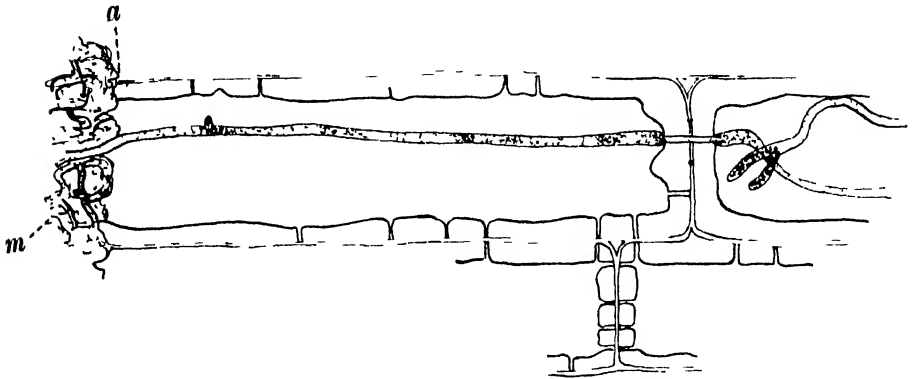


Fig. 10. Radial longitudinal section, showing a colourless hypha entering the cut end (*a*) of a medullary ray cell, after artificial infection of ash wood, and passing inwards into the next cell through a pit. *m*—mycelium outside the wood.

used to test the presence of lignin, ruthenium red and methylene blue the presence of pectin. These were found to occur in both colourless and brown woods, there being no complete disappearance of either substance in the infected wood.

From experiments, which will be described later, whereby ash wood was artificially infected with the spores of this fungus, the attack and development of the fungus in the wood could be understood. The fungus entered through the vessels, fibres or medullary rays at the cut end of a piece of wood. In the vessels, the hyphae on entering were very fine and colourless; they increased in number and became wider and tangled together; and they travelled down the vessel penetrating far into the wood. In the fibres, the hyphae passed straight down, rarely branching at first, but eventually branches were formed at right angles to the

original hypha, passing through the fibre pits into adjoining cells. In the medullary rays, the colourless hyphae travelled along the length of the ray, while some hyphae branched off and passed up and down the wood. These hyphae always travelled from one cell to another by means of the pits, thus permeating the whole tissue locally (Fig. 10).

With the ageing of the fungus the formation of black hyphae and the production of the brown colour ensue. In the artificial infections the black dichotomous hyphae were produced first near the cut end of the vessels, but eventually they occupied the whole length. They also were formed in the fibres and parenchyma cells, while the straight brown hyphae were produced in the medullary ray cells. Very soon after the hyphae had penetrated the wood, brown, amorphous, granular bodies were produced, most abundantly in the fibres and vessels, but also in the medullary

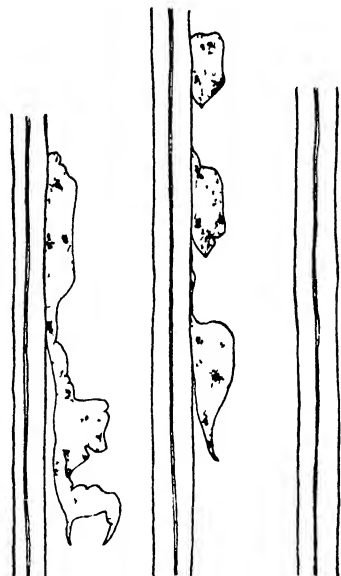


Fig. 11. Longitudinal section of fibres, showing granular bodies associated with artificial infection of ash wood.

ray cells and the wood parenchyma. It was concluded that the substance must be a product of decomposition of the nature of a "wound gum" as it was insoluble in acetone, alcohol, benzol, petrol ether, concentrated hydrochloric acid, nitric acid, sulphuric acid, potassium, ammonium and sodium hydroxide, but dissolved in a mixture of concentrated nitric and sulphuric acids. It did not stain with phloroglucin and hydrochloric acid, yet parts of it stained blue with lactic blue and picro-aniline blue (Fig. 11).

Several problems present themselves as to the fungus responsible. First, the identity of the fungus or fungi present had to be determined; second, the conditions of activity of the fungus in wood, as regards water, oxygen and acidity, including the conditions of infection and germination, and the nature of the food used by the fungus; third, the origin and nature of the brown substance.

For the purposes of identification and of determination of the conditions of growth, and the effect of the fungal attack, a number of cultures of the fungus were made.

## CULTURES.

(1) In order to induce the fungus to grow out of the wood, a piece of the infected wood was soaked in running water for six hours or more, dipped in 0.1 per cent. mercuric chloride in alcohol, and then kept damp in a potato dish. In about a fortnight, conidial tufts emerged, forming small, irregularly branched, upright, pale pink hummocks, covered with spores. These tufts never grew out of the blackened spring vessels, but only from the colourless or brown summer wood.

The structure of these conidiophores and conidia agreed with that of *Daldinia concentrica*. Consequently, fruit-bodies of that fungus connected with the wood of living ash trees were obtained, so that the ascospores might be used for cultures.

(2) Small pieces of wood were sterilised by steam in large plugged test-tubes and a suspension of either the conidia or the ascospores in sterile, distilled water was added to the tubes. The woods used were: sapwood of sycamore, hornbeam, oak (conidia only); heartwood of mulberry, elm, walnut, pine (conidia only), larch and American birch (ascospores only); the ripewood of beech.

(3) The following synthetic media were infected with conidia and ascospores: (a) *solid media*: agar: plain, 2 per cent. peptone, 2–20 per cent. sucrose, 5–10 per cent. malt, 2 per cent. turnip, 2 per cent. potato, 2 per cent. prune, and 1 per cent. starch; gelatine: 2 per cent. bouillon, and 2 per cent. peptone; cotton wool + a solution of 1 per cent.  $\text{KNO}_3$ , 0.5 per cent.  $\text{KH}_2\text{PO}_4$ , 0.2 per cent.  $\text{MgSO}_4$ ; (b) *liquid media*: tap water, distilled water, and solutions of different concentrations of malt, and sucrose; 2 per cent. galactose, 5 per cent. glucose, gum arabic, 1–5 per cent. starch, 0.5 per cent. onion pectin, 0.5 per cent. Citrus pectin, 0.5 per cent. beech hemicellulose B, 3 per cent. dextrose, 3 per cent. inulin, 2 per cent. xylose, 2 per cent. arabinose, 2 per cent. levulose, 2 per cent. maltose, 2 per cent. mannite and 0.5 per cent. lichenin.

(4) In order to show whether the spores needed an alkaline, acid or neutral medium in which to germinate, conidia and ascospores were placed in sucrose solutions containing different percentages of acid and alkali. The acids used were citric and acetic, and the alkali was caustic potash.

## IDENTITY.

(1) The conidiophores constituting the hummocks emerging from the moistened wood were hyaline, irregularly branched, septate; the branches bore verticillate branches, at the tips of which were produced clusters

of stalked conidia; the conidia were colourless, ovoid, with the attachment end more pointed,  $6.5-8 \times 5-6 \mu$  in dimension. The type, as Bayliss Elliott (2) suggested, is evidently that of a *Botrytis* (Figs. 12, 13, 14, 15).

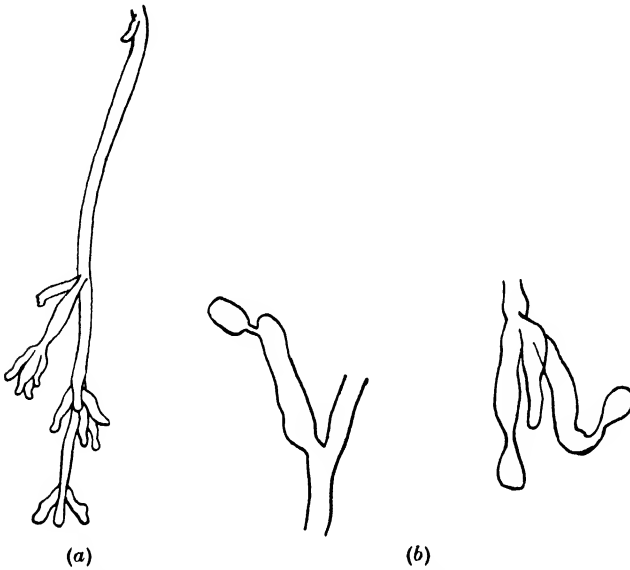


Fig. 12. (a) Conidiophore, and (b) verticillate branches bearing conidia emerging from moistened "calico-wood."

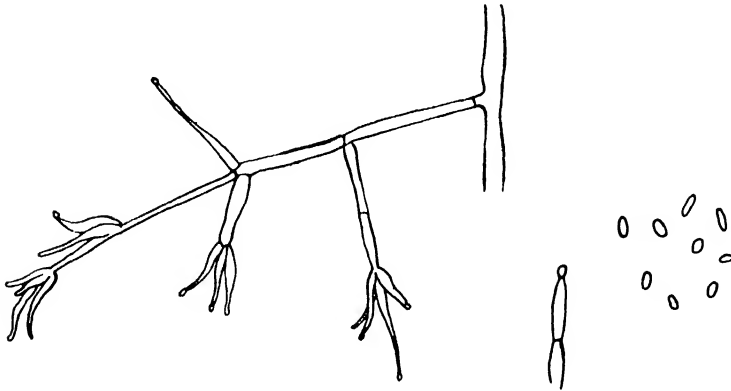


Fig. 13. Conidiophore and conidia of *D. concentrica* grown on bouillon gelatine.

This description agrees with those of *Daldinia concentrica* (Bolt.) Ces. et de Not. given by Tulasne in *Selecta fungorum Carpologie*, T. II, 1863; by Möller in "Phycomyceten und Ascomyceten," *Botanische Mittheil-*

*ungen aus den Tropen*, Schimper. Heft ix, p. 264, 1901; by Molliard, "Forme conidienne de *Daldinia concentrica*," *Bull. Soc. Myc. de France*, T. xx, pp. 55-60, 1904; by Bayliss Elliott in "On the formation of conidia and the growth of the stroma of *Daldinia concentrica*," *Trans. Brit. Myc. Soc.* vol. vi, pt III, 1920; and by others.

(2) The ash wood to which the ascocarps of *Daldinia concentrica* were attached showed to the naked eye brown streakings and black hyphae in the spring vessels. Under the microscope, the characteristic

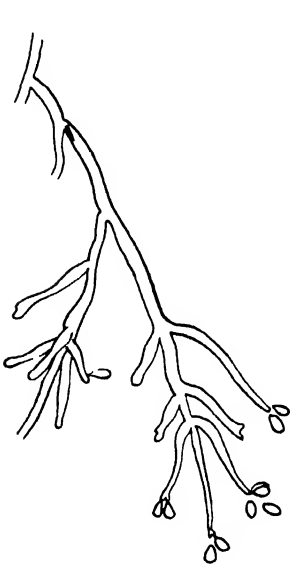


Fig. 14.

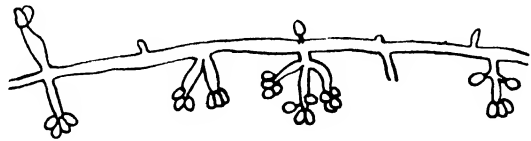


Fig. 15 (a).

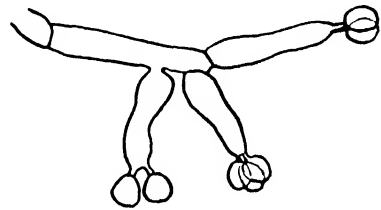


Fig. 15 (b).



Fig. 15 (c).

Fig. 14. Conidiophore and conidia produced on ash wood after artificial infection.

Fig. 15. (a) Conidiophore on malt agar, (b) part of same enlarged, (c) hypha in darkened malt agar turning brown at the tip owing to the change in colour of its contents.

dichotomously branched black hyphae and straight brown hyphae were observed in the wood and the bark, while colourless hyphae were present in nearly all the cells and vessels. Brown streaking was not so pronounced in this wood as it was in the first pieces examined, and occurred only in the bark and the adjacent wood.

(3) The ascocarps attached to the outside of this wood extruded ascospores in threads. When these fruit-bodies were kept in a damp atmosphere any ascospores adhering to them germinated and produced conidiophores and conidia identical with those emerging from the first piece of wood.

(4) The conidia used to infect the pieces of wood in the tubes germinated in all cases and produced mycelia. The mycelium was at first submerged in the water at the bottom of the tube or clung to the moist surface of the wood, but it soon began to penetrate the wood; and in a few weeks the blocks were covered on the outside with a mycelium bearing conidiophores and conidia, and were infected internally with hyphae. Colourless hyphae occurred in all the tissues of the wood, brown and black hyphae not being produced until later. Black dichotomous hyphae were produced on the outside of the wood before they were formed inside; and likewise, the liquid in the tube and the external tissues of the wood were turned brown long before any brown streaking could be seen inside the wood.

(5) Ascospores germinated and infected the wood blocks in precisely the same way, the mycelium and the brown colour produced agreeing in all cases with those produced by the conidia.

(6) Growth of the mycelium derived from the germination of conidia on agar and gelatine media took place on each of the different kinds mentioned before. On sucrose, malt, turnip, potato, prune and starch agar, *i.e.* carbohydrate agars, development was similar, resulting in all cases in a mycelium bearing conidia and black dichotomous hyphae, and the blackening of the medium. On plain agar, bouillon and peptone gelatine, no black or brown colour was formed either in the medium or in the hyphae.

(7) The results of the growth of ascospores on agar and gelatine media were the same as those obtained for conidia.

(8) In all of the liquid media mentioned, both conidia and ascospores germinated and produced larger or smaller mycelia.

*In water* to which no food material had been added, both kinds of spores germinated and even produced small mycelia probably at the expense of the ungerminated spores. The hyphae so produced were colourless, shortly and irregularly segmented, while the ends of some of the hyphae bore verticillate branches with conidia, though these feeble conidiophores might be replaced by clumps of branches segmented into oidia: no aerial hyphae were formed (Figs. 16 and 17).

*In malt solutions* the submerged mycelium was similar to that just described, but the segments were longer: there was no sign of production of conidiophores, but the conidia were budded off from any submerged hypha and these spores could in turn bud like a yeast. When the mycelium was so large that part reached the surface of the liquid, a typical aerial mycelium was formed, with pink conidial hummocks,

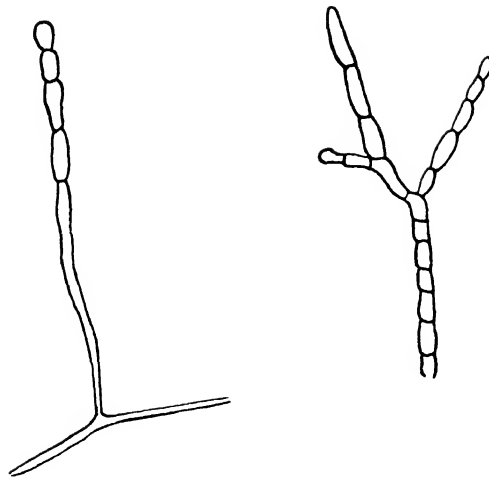


Fig. 16. Oidial form of hyphae submerged in water.

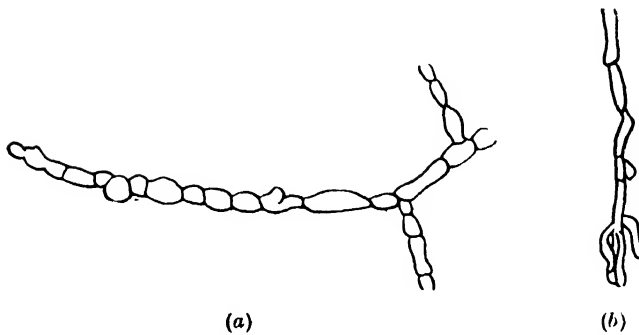


Fig. 17. (a) Short-segmented hypha, and (b) conidiophore, submerged in water.

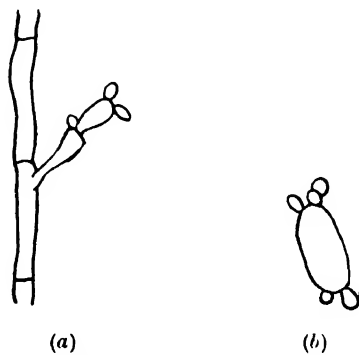


Fig. 18. (a) Hypha bearing spores, and (b) budding spore, submerged in malt solution.

conidiophores and black dichotomous hyphae. The hyphae just below the surface and the top part of the malt solution became dark brown in colour (Fig. 18).

*In sucrose solutions* there was still further differentiation of hyphae. After several months growth, the submerged mycelium and the liquid were dark brown; and the hyphae were of three kinds: (1) very dark brown to black, wide, short-segmented and sometimes monstrous in width; (2) extremely fine, colourless or with patchy brown contents, and long-segmented; (3) intermediate between (1) and (2) and often including segments swollen into vesicles (Fig. 19).

*Other solutions.* Growth was fair in beech hemicellulose and onion pectin solutions but was extremely feeble in Citrus pectin solution. The mycelium developed well in all the other solutions, and a brown colour was produced in all except inulin, mannite, Citrus and onion pectin, and beech hemicellulose.

*Conclusion.* These cultures prove that one species of fungus, *Daldinia concentrica*, alone produced the symptoms in the original sample of "calico-wood."

#### DEVELOPMENT OF FUNGUS.

On carbohydrate agar, the *conidium* germinated in a day or two by producing a germ-tube which emerged at one end or from the side and branched soon, or not until after the formation of the first septum. Branching and growth continued until a circular mat of creeping, colour-

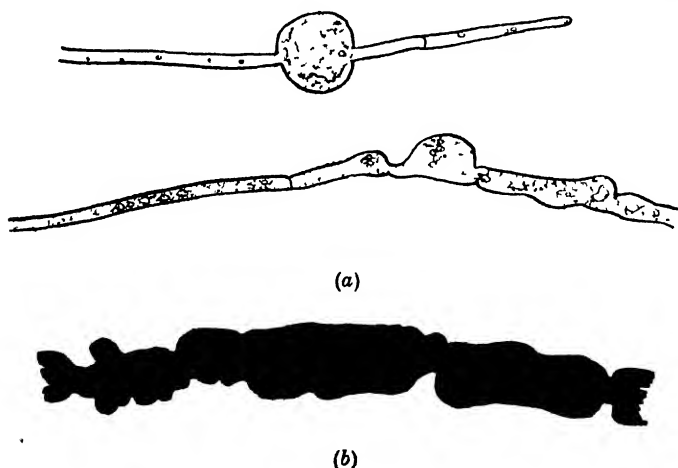


Fig. 19. (a) Hyphae bearing vesicles, (b) monstrous black hypha, submerged in sucrose solution.



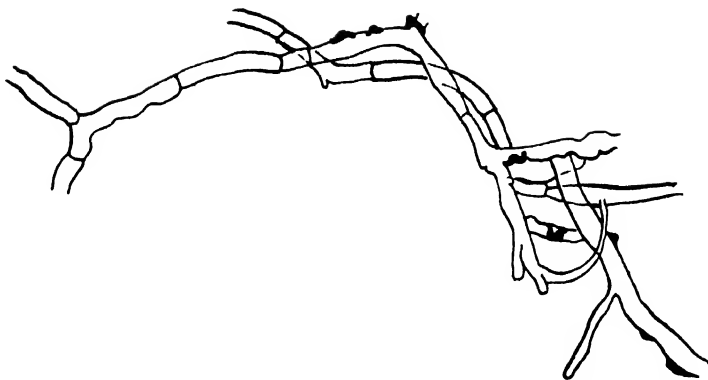


Fig. 20. First stage in development of black dichotomous mycelium—brown pigment beginning to be deposited, outside colourless hyphae, in small drops.

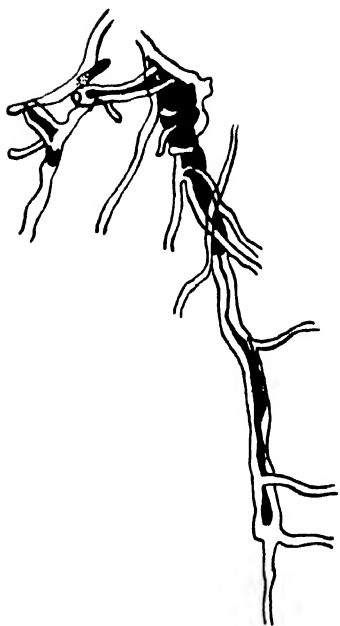


Fig. 21.

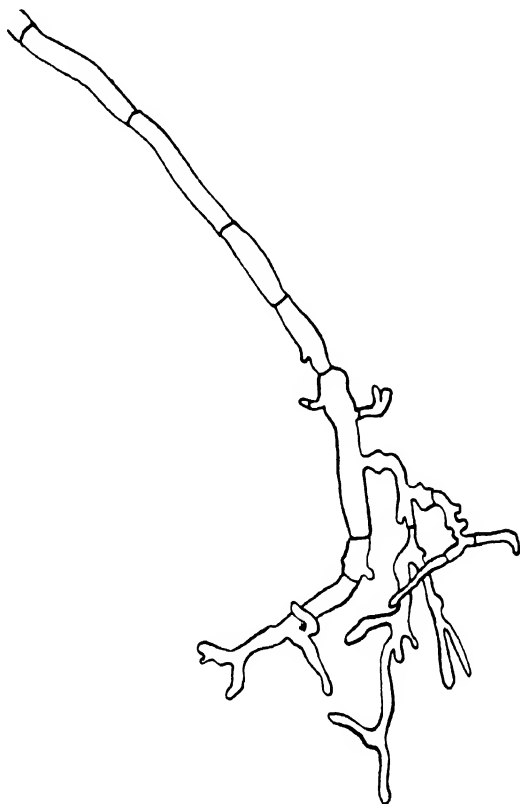


Fig. 22.

Fig. 21. Later stage, increasing amount of external black pigment.

Fig. 22. Dichotomous branches from colourless hypha.

less, septate hyphae was formed. A week after sowing, the mycelium was white and fluffy, due to the presence of aerial hyphae which eventually became the conidiophores. After the mycelium was well-grown, darkening of the agar took place; this started in the centre, *i.e.* under the oldest part of the mycelium, and spread outwards until all the agar was changed from normal yellow to dark brown or black. This darkening of the agar seems to correspond to the brown streaking in the ash wood.

At the same time new types of mycelia were produced. These were minute, spherical, black bodies, about  $300\mu$  in diameter, consisting of matted dichotomous hyphae identical with those described as occurring in the wood vessels. The origin of these bodies was as follows: colourless branches arose from the original creeping hyphae, which forked dichotomously; a brown pigment was deposited outside the branches and held in oil-like drops on the hyphae. This pigment either at once diffused uniformly throughout the cell-wall of the hyphae or remained outside in droplets for some time. The branches increased in number, continuing to branch dichotomously, until the mature body was formed, in which the hyphae were black and without contents and often had black drops of pigment outside. No colourless hyphae with contents, nor spores, were associated with these bodies, so that they could not have been sclerotia. These bodies arose first in the centre of the mycelium and later on others arose nearer to the periphery; they were aerial, or partly or wholly embedded in the agar (Figs. 20-23).

Ordinary creeping hyphae embedded in the blackened agar became brown owing to the change in colour of their contents.

A sickly sweet smell was always produced by the fungus on agar medium.

The *ascospore* germinated, as figured by Molliard (6), by splitting of the outer layer of the wall down one side; the brown outer membrane was pushed aside by a hyaline swelling which gave rise to the germ-tube

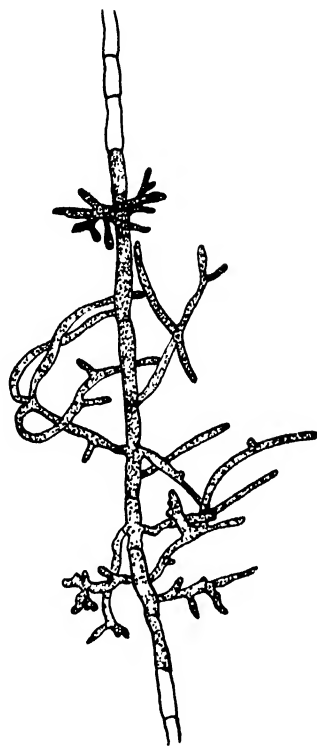


Fig. 23. Brown dichotomous hyphae branching off an originally colourless hypha.

which branched very soon to produce the creeping hyphae. Subsequently the typical conidiophores and black dichotomous mycelia were formed and the agar medium was turned dark brown.

The significance of the black dichotomous mycelia is unknown, although they are produced so abundantly. Molliard<sup>(6)</sup> describes how they are produced when the mycelium growing on carrot in a test-tube comes in contact with the glass, which suggests that they are due to desiccation. This can hardly be the case since they are produced also in liquid media and embedded in agar media. They might be compared with the brown hyphae temporarily formed by *Armillaria mellea* (*Agaricus melleus*) described by Hartig<sup>(3)</sup> in the wood of its host, since both kinds lose their protoplasmic contents, while those of *Armillaria* also lose their walls and are dissolved by the tree; and both might possibly function as temporary food reservoirs or excretory organs. Another suggestion is that the black mycelium represents an arrested development of ascocarps, and is therefore comparable with the sterile yellow mycelium (representing an arrested basidiocarp) of *Lenzites* outside wood.

#### EXTERNAL CONDITIONS OF GROWTH.

##### 1. *Water supply.*

The following facts suggest that this fungus demands a considerable amount of water in the wood:

(1) *Daldinia concentrica* is one of the ascomycetes, the wood-attacking members of which generally need a considerable supply of water in the wood.

(2) The vegetative mycelium grows exclusively inside the wood, only the conidiophores and ascocarps being produced externally.

(3) A submerged mycelium can be formed and live quite well in various liquids.

(4) When a rod of ash wood was placed in a plugged test-tube with water at the bottom, and infected with either the spores or mycelium of the fungus, there were most hyphae in the wet wood above the liquid, the amount decreasing downwards in the totally submerged wood and upwards in the drier wood. This demand for water harmonizes with the fact that the fungus grows in the sapwood of the ash.

## 2. Oxygen.

The fact that the mycelium can grow submerged in aqueous solutions suggests that a feeble supply of oxygen suffices at least for the development of the vegetative mycelium. Attempts were made to cause the spores to germinate in anaerobic conditions, *i.e.* in sealed tubes of aqueous malt solution or malt agar, but with no result, so apparently a certain amount of oxygen is necessary for germination. No experiments were conducted on the resistance to carbon dioxide.

## 3. Alkalinity and acidity of the medium.

As both ascospores and conidia were able to attack and penetrate sound wood, it was surmised that they did not need for germination an acid medium. The results of cultures where the spores were placed in sucrose solutions containing different percentages of citric acid, acetic acid and caustic potash are given in Table I, the presence of *a* in a column of percentages indicating that *ascospores* were able to germinate in that percentage, of *c* that *conidia* germinated; both kinds of spores were tried in all the percentages mentioned, but they germinated only in those marked.

Table I.

Acid							Neutral							
.12	.10	.08	.06	.04	.02	.01								
—	—	—	—	—	—	<i>c</i>	<i>c</i>	<i>c</i>	Citric acid					
—	—	—	—	—	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>						
—	—	—	<i>c</i>	<i>c</i>	<i>c</i>	<i>c</i>	<i>c</i>	<i>c</i>	Acetic acid					
—	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>						
—	—	—	—	—	—	—	<i>c</i>	<i>c</i>	Caustic potash					
—	—	—	—	—	—	—	<i>a</i>	<i>a</i>						
Alkali														
.02	.04	.06	.08	.10	.12	.14	.16	.18	.20	.22	.24	.25	.26	
—	—	—	—	—	—	—	—	—	—	—	—	—	—	Citric acid
—	—	—	—	—	—	—	—	—	—	—	—	—	—	acid
—	—	—	—	—	—	—	—	—	—	—	—	—	—	Acetic acid
—	—	—	—	—	—	—	—	—	—	—	—	—	—	acid
<i>c</i>	<i>c</i>	<i>c</i>	<i>c</i>	<i>c</i>	<i>c</i>	—	—	—	—	—	—	—	—	Caustic
<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>	—	potash

Both ascospores and conidia germinated in greater concentrations of alkalinity than of acidity, and in the case of the two acids and the alkali the ascospores withstood twice the strength that was toxic to the conidia.

These results contrast well with those given by Möller(5) for the germination of spores of *Merulius lacrymans* (*domesticus*). Dealing with

inorganic acids in distilled water, he obtained germination in 0.001 per cent. sulphuric acid, in 6 per cent. phosphoric acid (commercial acid, 25 per cent.  $\text{H}_3\text{PO}_4$ ) and in 0.25 per cent. commercial hydrochloric acid plus 0.06 per cent. malt. Using organic acids, the optimum percentage for germination was 5 per cent. in the case of malic, citric and tartaric acids, 1 per cent. for oxalic acid, and with lactic acid germination took place in concentrations ranging from 10 per cent. to 0.001 per cent. He also attempted to germinate the spores in 10 per cent. to 0.0005 per cent. potash, but with no result. Thus the *optimum* for germination of *Merulius* in the case of citric acid was 5 per cent., but the *maximum* for *Daldinia* was below 0.04 per cent.; and whereas the spores of *Merulius* will not germinate in a solution of caustic potash containing 0.0005 per cent., the ascospores of *Daldinia* will do so in a solution of 0.25 per cent., the conidia in one of 0.12 per cent. Thus the spores of *Merulius lacrymans* need an acid medium and do not attack sound wood in the absence of an acid, while the spores of *Daldinia*, which lives either as a saprophyte or as a parasite on *Fraxinus* or *Alnus*, prefer a neutral medium, and can attack sound wood.

#### FOOD MATERIAL OF FUNGUS.

As the microchemical reactions of the lignified walls gave no clue to the food material used by the fungus, cultures were made on various nutrient media to determine what food materials it was capable of utilising.

At the same time observations were made on the production or non-production of the brown substance.

##### 1. *Progress of mycelium on nutrient media.*

Various kinds of wood were used on which to grow the fungus, and included the sapwood of sycamore, oak, hornbeam and ash; the heartwood of mulberry, pine, larch, elm, walnut and American birch; and the ripewood of beech. The fact that the fungus lived on these heartwoods proves that it must be independent of starch, sugar and protein. At the same time, its power of dealing with the wood walls as regards the main structure is feeble, as shown by the firmness of the wood, passage of hyphae solely through the pits, and no change in lignin or pectin reactions.

Proof that the fungus can deal with starch is given by the wood cultures and by starch media. In the rod of ash wood infected with fungus in a test-tube, already described, both the relative amounts of

hyphae and of starch were determined, and it was clear that where there were most hyphae there was least starch. Moreover, after the fungus had been growing on starch agar and also soluble starch in water for some time, these were tested, for starch (with iodine) and for sugar (Fehling's solution), but both were absent.

That the fungus could grow in and utilise as food other substances is shown in Table II, which records the growth and presence or absence of the brown substance in media containing different carbohydrates<sup>1</sup>.

Cotton wool with the addition of inorganic salts supported the fungus; the cotton eventually became brown and the strands decomposed or eaten away in places.

Table II.

Substance used by fungus as food	Growth	Colour
Solution in water:		
(1) Sugars		
Pentoses		
Arabinose 2 %	Fair	Pale brown
Xylose 2 %	Moderate	Pale brown
Hexoses		
Dextrose 3 %	Fair	Brown
Levulose 2 %	Good	Brown
Galactose 2 %	Good	Brown
Disaccharides		
Sucrose 2-20 %	Good	Brown
Maltose 5 %	Good	Brown
(2) Polysaccharides		
Starches		
Potato starch 1-5 %	Good	Brown
Inulin 3 %	Good	○
Lichenin 0.5 %	Good	Brown
Pentosanes		
Beech hemicellulose B 0.5 %	Moderate	○
Pectic bodies		
Onion pectin 0.5 %	Good	○
Citrus pectin 0.5 %	Feeble	○
Compound substances:		
Agar, plain	Poor	○
Agar, carbohydrate 2-20 %	Good	Brown-black
Agar, peptone 2 %	Moderate	Pale brown
Gelatine, peptone	Fair	○
Gelatine, bouillon	Fair	○
Wood, Ash	Good	Brown
Cotton wool + solution of		
1 % $\text{KNO}_3$	Good	Brown
0.5 % $\text{KH}_2\text{PO}_4$		
0.2 % $\text{MgSO}_4$		

<sup>1</sup> The presence of a ○ in the table indicates the absence of the brown colouring matter.

## 2. Nature and production of brown substance.

The brown substance produced both in wood and in synthetic media, solid and liquid, was insoluble in the following reagents: acetone, alcohol, benzol, petrol ether, concentrated sulphuric, hydrochloric and nitric acids, and strong potassium, ammonium and sodium hydroxides whether used hot or cold. The only positive reaction that was obtained, was a change in colour from brown to bottle-green in the presence of any alkali, strong or weak, and a very slight change to reddish-brown with acids. These changes were reversible, for if the material were neutralised again, the original brown colour returned. The tests were made on the streaked wood, brown agar and brown liquid culture media, hyphae with brown contents and brown dichotomous hyphae, with the same results in each except in the case of the brown hyphae, which only gave a positive reaction when young (on agar) and not when old, as *e.g.* inside wood or constituting a fruit-body.

With regard to the origin of the brown substance, it seems likely that it is formed directly or indirectly from the decomposition of carbohydrates. From the table above it can be seen that pentoses, hexoses, disaccharides and polysaccharides all yielded a brown colour. Although the fungus grew in water or on plain agar and proteins, no brown colour was produced, neither was it produced in inulin, pectic bodies nor the pentosane beech hemicellulose. The cultures showed that the amount of coloured substance produced was not proportional to the vigour of growth, but was determined by the composition of the nutrient medium. It therefore seems probable that in the sapwood of ash, either the starch or sugar in the cell-contents or some carbohydrate in the cell-walls is decomposed, and the brown colouring matter so formed stains the walls and the contents of the lumina, or that both these processes take place with the same result. In one case it was seen that the brown colour was confined to the middle lamella in immediate continuity with the pit-closing membrane. Moreover in a number of cases, the colouring body present was confined to the cavities of the pits. These facts suggest that the attack on the wall takes place first, *via* the pits, on the middle lamella immediately surrounding the pit-membrane. There are a number of cases known in which, according to Hartig(4), the middle lamella is particularly attacked, with the result that the wood-constituents become isolated (*e.g.* *Pinus sylvestris* and *Trametes pini*, *Quercus* and *Thelephora perdix* (*Stereum frustulosum*)).

Again it is possible that this brown substance is allied to humus.

Wehmer(8) in a paper "Lignin and Humin substances in the fungous decay of wood" draws attention to the fact that a substance rich in carbon is produced in wood attacked by *Merulius lacrymans*, and that the same substances are extracted from *Merulius* wood as from peat, suggesting that lignin in wood may be decomposed to form humin, a fact which Schorger(7) substantiates when dealing with the fermentation of wood by filamentous fungi.

#### SUMMARY AND CONCLUSION.

(1) Calico-wood (ash wood) was found to be infected with hyphae of two kinds: (a) black, dichotomously branched, and (b) colourless. In certain patches, the wood walls and cell-contents were stained brown.

(2) Conidia, obtained by keeping some of the wood moist, and ascospores, taken from the ascocarps of *Daldinia concentrica*, were grown on various solid and liquid media. The mycelia and the discoloration of the medium resulting from the germination of the conidium agreed with those resulting from the germination of the ascospore and with the hyphae and brown staining in the calico-wood.

(3) The morphology and development of the mycelium in the wood, the results of the growth in culture of the conidia and ascospores, and the comparison between calico-wood and ash wood attacked by *Daldinia concentrica*, proved that the fungus in calico-wood was *D. concentrica*.

(4) The external conditions of growth as regards water, oxygen and alkalinity and acidity, the food materials utilised by the fungus inside the wood, and the nature and production of the brown substance were investigated. Certain facts suggest that the attack in the wood begins in the middle lamella in contact with the pits.

(5) Calico-wood is therefore the wood of *Fraxinus excelsior* infected with *Daldinia concentrica*.

My thanks are due to Prof. Percy Groom, F.R.S., for his suggestions and helpful criticism, and to the Forestry Commissioners for their assistance in securing specimens of ash from the standing tree attacked by *Daldinia concentrica*.



## REFERENCES.

- (1) BARY, DE (1884). *Comparative Anatomy of the Phanerogams and Ferns*.
- (2) BAYLISS ELLIOTT, JESSIE S. (1920). On the formation of conidia and the growth of the stroma of *Daldinia concentrica*. *Trans. Brit. Myc. Soc.* vi, pt III, 269.
- (3) HARTIG, R. (1894). *The Diseases of Trees*, pp. 211–212.
- (4) — (1894). *Ibid.* pp. 191 and 203.
- (5) MÖLLER, A. (1912). Die Meruliusfäule des Bauholzes. *Hausschwammforschungen*, VI, Teil II, 260.
- (6) MOLLIARD (1904). Forme conidienne de *Daldinia concentrica*. *Bull. Soc. Myc. de France*, XX, 55–60.
- (7) SCHORGER, A. W. (1926). *The Chemistry of Cellulose and Wood*, pp. 482–495.
- (8) WEHMER, C. (1927). Lignin und Huminstoffe bei der pilzlichen Holzzersetzung. *Ber. Deutsch. Bot. Gesellsch.* XLV, 8, 536–539.

(Received December 6th, 1928.)

## ADDITIONAL HOSTS OF *SYNCHYTRIUM* *ENDOBIOTICUM* (SCHILB.) PERC.

By MARY S. MARTIN, B.Sc.

(Department of Mycology, Rothamsted Experimental Station, Harpenden.)

(With Plates XVIII and XIX.)

### INTRODUCTION.

IN connection with the investigations on Wart Disease of Potato being carried out in the Rothamsted Experimental Station it was found necessary to test the infectivity of *Synchytrium endobioticum* on a number of additional species of plants. In selecting plants for this experiment, it was considered advisable to include not only such species of the Solanaceae as occur in the British flora and in cultivation in this country, but also certain plants which are weeds and crops of other countries in which *Synchytrium endobioticum* has been recorded.

Four species of American weeds were selected, *Solanum Commersonii* and *Solanum Jamesii* to represent the tuber bearing class; *Solanum nodiflorum* and *Nicandra physalodes* among the non-tuber bearing weeds. With the exception of *Solanum nodiflorum*, these species have been tested in America and *Solanum Commersonii*(7) and *Solanum Jamesii*(7) found susceptible to wart disease, while infection has not been recorded in *Nicandra physalodes*.

Among the British solanaceous weeds *Solanum nigrum*, *Solanum dulcamara*—including a white variety *Solanum dulcamara alba*—*Datura stramonium* and *Hyoscyamus niger* were chosen. Of these *Solanum nigrum*(2), *Solanum dulcamara*(2) and *Hyoscyamus niger*(3) have previously been found susceptible and were included rather in the nature of controls.

*Solanum dulcamara* var. *villosissimum* and *Solanum villosum* are common continental weeds. *Solanum villosum* is occasionally found in this country on waste ground in the neighbourhood of docks. *Petunia violacea*, *Petunia alba*, *Salpiglossis sinuata*, *Nicotiana affinis* and *Nicotiana Sanderae* are members of the Solanaceae which are commonly met in garden cultivation in this country. As examples of crop plants tobacco, tomato, egg plant and winter cherry were chosen. Of the above infection has been recorded in tomato(7) and suspected in *Salpiglossis*.

## CULTIVATION OF EXPERIMENTAL PLANTS.

The plants were all raised and grown in a warm glasshouse throughout the experiments. The seed was sown in clean soil, and as soon as the young plants were large enough to handle, they were transplanted into pots of seven inches diameter. The compost was made of fresh potting soil well mixed with one half its bulk of soil contaminated with *Synchytrium endobioticum*. Previous workers (4, 5), investigating the conditions for infection of the potato by *Synchytrium*, have found that a certain period of excessive moisture in the soil results in a greater percentage of infection. In order to maintain these conditions, the pots were not crocked in the usual manner but cavings or sphagnum moss was used instead, so that there might be a free upward passage of water from the saucers in which the pots stood. The seedlings were treated normally until they were well established, *i.e.* from seven to fourteen days after transplanting, then earthenware saucers were placed under the pots and the amount of water supplied was increased so that some water always remained in the saucers.

Although the plants were grown under these conditions of practically water-logged soil until the experiments were concluded they grew well, with very few exceptions, and looked particularly healthy —flowering and fruiting as satisfactorily as under more normal conditions.

## METHODS OF INOCULATION.

(a) *Soil inoculation.*

Decaying potato warts were finely ground and mixed with the compost. All the plants were grown in this contaminated soil which was kept very moist to give the most suitable conditions for the germination of the contained winter sporangia. In susceptible potatoes rapid and profuse infection is usually obtained in the collar region. A small amount of very highly contaminated soil (ground wart mixed with a little sand) was therefore placed around the collars of the experimental plants to increase the chance of infection in that area.

(b) *Glynne's "green wart" method.*

This method (5) depends on the more rapid and certain germination of the summer sporangia in a green wart in comparison with the resting sporangia contained in a black wart. The method consists of placing a piece of young and freshly formed wart in contact with the plant to be

## 424 *Additional Hosts of Synchytrium endobioticum*

inoculated. A film of water is maintained between the plant and the wart to ensure the correct conditions for infection, *i.e.* free water into which the zoospores from the summer sori may be discharged.

All the wart employed in these experiments was derived from Arran Chief potatoes. This variety is very susceptible to *Synchytrium endobioticum* and the warts are produced rapidly and in abundance on the young shoots. The warts were removed as soon as they were of a convenient size to handle, from 0.5 cm. in diameter upwards, and while of a pale yellow or green colour.

The actual procedure was as follows. A leaf axil on a plant was selected in which the young axillary shoot was just beginning to grow; about the third node from the ground level in most cases. A small wart or piece of wart was placed in contact with the young shoot and base of the leaf and then covered with moist sphagnum moss. The wart and moss were tied firmly in position with bast to ensure contact with the host plant. The sphagnum was moistened at regular intervals with water to keep the wart fresh and to preserve a film of water between the wart and young shoot.

In *Hyoscyamus niger* the wart was tied to one of the young leaves as the plants were in the sessile rosette form, not having produced a central stem.

The wart was left in contact with the shoot for varying periods and the plants were examined at intervals for signs of infection. The usual period of contact was three weeks, although certain plants showed warted outgrowths after fifteen days.

### (c) *Attempted localisation of infection by swarm spores.*

This method is a modification of Glynne's method and was tested on Arran Chief potatoes. The aim was so to localise the point of inoculation that the manifestation of the disease might be confidently looked for in a small circumscribed area, thus saving much tedious examination by lens and eye over a comparatively large area of the shoot.

Tubers were selected with a shoot about 1 cm. long. The latter was preferably situated at the rose end and developing somewhat obliquely outwards from the long axis of the tuber. The other shoots were rubbed off. Small glass cups were made with a minute aperture near the base and so placed that the aperture came in contact with the shoot. Each cup was held in position against a young shoot by brass wire. A small piece of green wart was put in the cup and a drop of water added to

ensure contact between the wart and the small area of tissue exposed through the aperture in the bottom of the cup.

The tubers were placed in an incubator in wire cages and the wart frequently moistened. The shoots were examined at intervals for infection by *Synchytrium* but in no case was infection obtained. Although the method was not successful in these tests there seems reason to consider that some slight modification in the technique might make this an extremely useful method.

#### EXAMINATION OF PLANTS.

##### *Infection from the soil.*

The plants were removed from the pots and the roots and collar carefully washed free of soil. They were then thoroughly examined and any suspected portions removed and hand sectioned.

##### *Infection by green wart method.*

The young shoot and leaves of the plant which had been in contact with the wart were minutely examined and any portions bearing the slightest visible excrescences were removed and fixed in Flemming's weak solution. In some cases, even where no roughness or blistering could be discerned, samples were removed and fixed, in the hope of finding the parasite present in the tissues without any outward manifestation of its presence. The material was microtomed and permanent preparations made. The results of the experiments are given in Tables I-III.

#### RESULTS.

These experiments on the testing of Solanaceous plants for susceptibility to *Synchytrium endobioticum* have resulted in the finding of several new hosts for this fungus. Previous work on the infectivity of *Solanum nigrum*, *Solanum dulcamara* and *Lycopersicum esculentum* has been confirmed, and the following new hosts are here recorded: *Solanum dulcamara* var. *villosissimum*, *Nicandra physalodes*, *Solanum dulcamara alba*, *Solanum nodiflorum* and *Solanum villosum*. Infection has not been obtained in *Datura stramonium*, *Hyoscyamus niger*, *Nicotiana affinis*, *Nicotiana Sanderae*, *Nicotiana Tabacum*, *Petunia violacea*, *Petunia alba*, *Physalis Francheti*, *Salpiglossis sinuata*, *Solanum Commersonii*, *Solanum Jamesii* and *Solanum melongena esculentum*.

The reaction to the *Synchytrium* varied widely in the different hosts. On the majority of the species a definite warty outgrowth developed.

## 426 *Additional Hosts of Synchytrium endobioticum*

The production of a wart however was not taken as a criterion for infection. The presence of the parasite, in any stage in the tissues of the plant, has been accepted as evidence of a positive infection of the plant by *Synchytrium*, and the species so infected has been recorded as susceptible to the disease. This was considered the best attitude to adopt with regard to the very slight infections noted on some of the species, for example *Solanum dulcamara* var. *villosissimum*, *Nicandra physalodes* and *Solanum dulcamara alba*, because, although the infection was so slight, the parasite appeared to be perfectly healthy and capable of further development. The *Synchytrium* was never observed to be in process of disintegration after its entry into the host cell had been effected (1). If the host reaction or the amount of outgrowth developed is taken as an indication of the susceptibility of the host (2), then tomato, which produces warts easily, can be regarded as the most susceptible species examined. With this host a varietal test was conducted, the "green wart" method being employed for inoculation purposes, and of the fourteen varieties tested, all with the exception of three, Buckley, Sutton's Every Day and Sutton's Maincrop, proved susceptible to *Synchytrium* (see Table II). Kondine Red produced the most rapid reaction to the infection, the warted areas on the leaves being from 1.0–1.5 cm. in length within fourteen days of inoculation. Little pegs of tissue of about 0.5 mm. in diameter and 1–2 mm. in height were observed on the leaflets of the variety of "Fillbasket." These occurred either singly or in groups of two or three. Microscopic examination showed them to contain the fungus in various stages of growth. This form of host reaction is presumably an early stage before the distortion becomes more general over the leaf. In most cases the leaves of the plants of the successfully inoculated varieties were visibly distorted by the development of the fungus. The test confirms the fact that susceptibility to the disease is widespread among the varieties of tomato commonly used in cultivation in this country.

The *Solanum* group can be classed next to the tomato in degree of susceptibility or amount of host reaction. In this group warts were not produced with the frequency and regularity shown by the tomato. A definite warted outgrowth was produced on the leaf in one plant of *Solanum nigrum*, *Solanum nodiflorum* and *Solanum villosum* respectively. The remainder of the infections in these three species and in *Solanum dulcamara alba* were not easily visible as the hypertrophy of the leaf tissues had only just begun.

In the remaining hosts, *Solanum dulcamara* var. *villosissimum* and *Nicandra physalodes*, the infections were not visible to the naked eye.

The leaf of the former, which was found to contain *Synchytrium* in various stages, showed no visible external symptoms but in microtome section appeared to be slightly thicker than the normal leaf. There was none of the usual distortion which accompanies infection as in the tomato.

The infection in *Nicandra physalodes* was extremely slight. A young parasite was discovered near the median vein of a young leaf which was still folded, but its detection was quite impossible without microscopic examination. The pad of cells in which the parasite was situated is normal tissue covering the vein and not the beginning of hypertrophy of the host tissues.

The duration of these experiments was generally from fifteen to twenty-one days, and never more than five weeks, from the time the green wart was placed in contact with the shoot until the shoot was removed for examination. It is possible therefore, had it been practicable to leave the plants for a longer period, that the host reactions would have developed to a greater extent and that mature resting sporangia would have been found more abundantly in the tissues.

A varietal test was conducted with tobacco, six varieties being used (see Table III) and the green wart method employed as previously described. The results of the experiment were entirely negative.

The green wart test on *Solanum Commersonii* and *Solanum Jamesii* was attempted too late in the season, the haulms reaching maturity and withering before results could be expected. These plants will be tested again during the coming season and it is hoped to confirm Weiss's results.

In all experiments there was a total failure of any infection of the plants from the soil which indicates that for experimental purposes the green wart method of shoot inoculation is the more reliable. It is difficult to suggest any feasible explanation of the lack of infection from soil inoculation.

#### SUMMARY.

Infection of numerous species of Solanaceae by *Synchytrium endobioticum* has been obtained using Glynne's "green wart" method. Plants grown in contaminated soil did not show infection. The following new hosts are recorded: *Solanum dulcamara* var. *villosissimum*, *Nicandra physalodes*, *Solanum dulcamara* alba, *Solanum nodiflorum* and *Solanum villosum*.

In certain hosts the fungus may occur in the tissues with little or no external sign of its presence.

# 428 *Additional Hosts of Synchytrium endobioticum*

Table I.

*Infectibility of different species of host plant.*

Species	No. of plants inoculated	No. of plants infected	
		(1) Contaminated soil	(2) Green wart method
<i>Datura stramonium</i>	13	—	—
<i>Hyoscyamus niger</i>	4	—	—
<i>Solanum dulcamara</i> var. <i>villosissimum</i>	5	—	1
<i>Lycopersicum esculentum</i>	47	—	27
<i>Nicandra physalodes</i>	13	—	1
<i>Nicotiana affinis</i>	4	—	—
<i>Nicotiana Sanderae</i>	4	—	—
<i>Nicotiana Tabacum</i>	22	—	—
<i>Petunia violacea</i>	4	—	—
<i>Petunia alba</i>	4	—	—
<i>Physalis Francheti</i>	12	—	—
<i>Physalis</i> (Red fruit var.)	4	—	—
<i>Salpiglossis sinuata</i>	10	—	—
<i>Solanum Commersonii</i>	4	—	No test
<i>Solanum dulcamara</i>	2	—	1
<i>Solanum dulcamara alba</i>	4	—	1
<i>Solanum Jamesi</i>	4	—	No test
<i>Solanum melongena esculentum</i>	8	—	—
<i>Solanum nigrum</i>	4	—	2*
<i>Solanum nodiflorum</i>	8	—	4*
<i>Solanum villosum</i>	4	—	1*

\* One visible wart.

Table II.

*Tomata. Varietal test.*

Variety	No. of plants inoculated.	No of plants infected
	Green wart method	
A 1	3	1
Ailsa Craig	2	1
Best of All	3	3
Blaby	2	1
Buckley	2	—
Comet	2	1
Earliest of All	3	1
Fillbasket	2	1
Kondine Red	16	14
Manx Marvel	2	1
Princess of Wales	3	2
Sutton's Early Market	3	1
Sutton's Every Day	2	—
Sutton's Maincrop	2	—





Fig. 1

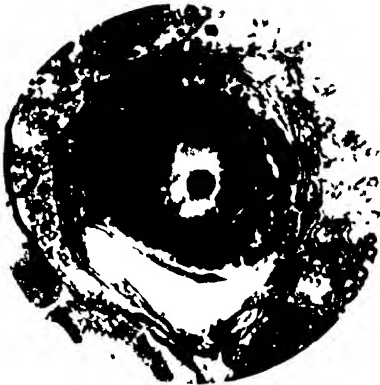


Fig. 2



Fig. 3

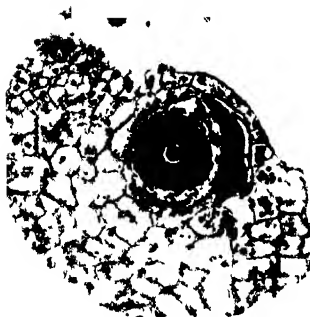


Fig. 4



Fig. 5





Fig. 1

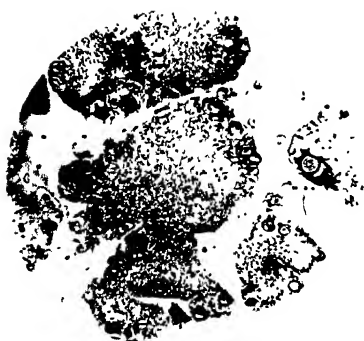


Fig. 2



Fig. 3



Fig. 4



Fig. 5



Fig. 6



Table III.

*Tobacco. Varietal test.*

Variety	No. of plants inoculated. Green wart method	No. of plants infected
Connecticut	3	—
Kentucky	3	—
Kentucky White Stem	3	—
Maryland	3	—
Virginia	3	—
White Burley	7	—

I am indebted to Dr W. B. Brierley for helpful criticism and suggestions received during the course of this work; and to the Director, Research and Experimental Station, Cheshunt, Herts, The Director, Royal Botanic Gardens, Kew, Surrey, The Regius Keeper, Royal Botanic Gardens, Edinburgh, and Martin H. Sutton, Esq., Erleigh Park, Whiteknights, Reading, for seed and tubers used in the experiments.

## REFERENCES.

- (1) CARTWRIGHT, K. (1926). On the Nature of the Resistance of the Potato to Wart Disease. *Ann. of Bot.* XL, 158, 391.
- (2) COTTON, A. D. (1916). Host Plants of *Synchytrium endobioticum*. *Kew Bull. Misc. Inform. London*, x, 272-275.
- (3) ESMARCH, F. (1925). Nachtschattengewächse als Wirtspflanzen des Kartoffelkrebspilzes (*Synchytrium endobioticum*). *Angew. Bot.* VII, 2, 108-120.
- (4) — (1927). Untersuchungen zur Biologie des Kartoffelkrebses. II. *Ibid.* IX, 2, 88-124.
- (5) GLYNNE, M. D. (1925). Infection Experiments with Wart Disease of Potatoes (*Synchytrium endobioticum* (Schilb.) Perc.). *Ann. App. Biol.* XII, 1, 34-60.
- (6) KOHLER, E. (1924). Ueber die Beziehungen des Kartoffelkrebsesregers (*Synchytrium endobioticum* (Schilb.) Perc.) zu seiner Wirtspflanze. *Centralbl. für Bakt.* 2, LXI, 1-4, 32-37.
- (7) WEISS, F. (1925). The Conditions of Infection in Potato Wart. *Am. Journ. Bot.* XII, 7, 413-443.

## EXPLANATION OF PLATES XVIII AND XIX.

## PLATE XVIII.

- Fig. 1. Winter sporangium of *Synchytrium endobioticum* in leaf tissue of *Solanum nigrum*.  
 Fig. 2. Young winter sporangium in leaf tissue of *Solanum dulcamara*.  
 Fig. 3. Five summer sporangia in a sorus in warted leaf of Tomato, variety Manx Marvel.  
 Fig. 4. Young winter sporangium in warted leaf of Tomato, variety Best of All.  
 Fig. 5. Young sporangia in warted leaf of Tomato, variety Kondine Red.

## PLATE XIX.

- Fig. 1. Sporangia in various stages of development in leaf tissues of *Solanum nodiflorum*.  
 Fig. 2. Warted leaf of *Solanum nodiflorum* showing sporangia in various stages of development.  
 Fig. 3. Sporangium in leaf tissues of *Solanum dulcamara* var. *villosissimum*.  
 Fig. 4. Young sporangium in leaf tissues of *Nicandra physalodes*.  
 Fig. 5. Young sporangium in leaf of *Solanum dulcamara alba*.  
 Fig. 6. Resting sporangia in warted leaf of *Solanum villosum*.

*Addendum.* Since going to Press infection has been obtained on *Solanum Jamesii*.

(Received February 27th, 1929.)

## THE EFFECT OF COPPER SULPHATE ON TOMATO PLANTS

BY O. OWEN, M.Sc., Ph.D., A.I.C.

(*Experimental and Research Station, Cheshunt, Herts.*)

COPPER in various forms has by long use become an established constituent of many fungicides and is the basis of several proprietary preparations recommended for the control of some of the fungi which affect tomato plants. Some years ago Dr Bewley introduced the mixture known as Cheshunt Compound<sup>1</sup> as a control for "damping off" of tomato seedlings, a disease which, until then, had exacted considerable toll of most nurseries during the propagating periods. Unfortunately, the success of this preparation has tended to lead to its abuse and nurserymen in various parts of the country have used it so liberally as to render the possibility of copper poisoning a real one. Accordingly, the experiments recorded here were carried out to obtain information regarding the effects of copper on tomato plants at various stages of growth. The work was arranged to provide information regarding the effect of copper on:

- (A) Germination.
- (B) Young plants.
- (C) Mature plants.

Experiments were also carried out to determine the effect of certain manures on the toxic effects due to copper in the soil.

Most of the experiments were repeated at different times in each of four years. Comparison of the results shows clearly that temperature, humidity and light intensity are factors which influence the results. An attempt to determine the significance of these individual factors was beyond the intention and scope of the present work and consequently the conclusions reached must be regarded as indicative rather than final. They are, however, likely to be of use to commercial growers.

<sup>1</sup> Cheshunt Compound consists of an intimate mixture of ammonium carbonate (11 parts) and copper sulphate (2 parts). The mixture is dissolved at the rate of 1 oz. in 2 gallons of water, and the solution watered on to the soil.

EFFECT OF COPPER SULPHATE ON THE GERMINATION OF  
TOMATO SEEDS.

In these experiments the soil was always air-dried before mixing with the salt and contained 2 or 3 per cent. of water. The salt was added either as powder and well mixed with the soil by hand or as a solution sprayed on to the soil, the whole being turned continually to ensure thorough mixing. After mixing the soil was damped to make it reasonably moist and allowed to stand for 24 hours before sowing. For the actual germination the ordinary seed boxes were used and 54 seeds sown in each box. The varieties Manx Marvel, Comet and E.S. 1 were used at different times and there appeared to be little difference in the results for the different varieties.

In Table I are shown *average* figures obtained in May 1927 for several batches of seeds. Seeds sown in October of the same year gave similar results, but germination was somewhat retarded throughout owing to the unfavourable weather conditions.

Table I.

Copper added as sulphate; % in soil	Percentage germination					
	5 days	6 days	11 days	15 days	18 days	22 days
0.0 (Controls)	15	20	30	100	100	100
0.1	50	55	55	80	80	80
0.3	65	95	95	95	95	95
0.5	25	45	45	70	70	70
0.75	15	25	35	45	45	45
1.0	0	0	0	0	20	20
2.0	0	0	0	0	45	45
3.0	0	0	0	0	0	0

Except in the controls the poison effects were apparent before the true leaves had developed. Even at the lowest concentration given in the table seedlings were very weakly and the cotyledons were of a dark colour. At the higher concentrations the seedlings made no growth and the root action was very restricted in all cases. The table suggests that the rate of germination is accelerated by the copper at a concentration up to 0.3 per cent. in the soil. The total germination, however, appears to be unaffected at this concentration.

A considerable amount of germination work was carried out between March and July 1928 in soils containing from 0 to 0.5 per cent. of copper. In all cases the inclusion of copper accelerated the rate of germination as already indicated in Table I. As before, however, the total germination

## 432     *Effect of Copper Sulphate on Tomato Plants*

was unaffected and in all cases up to a copper content of 0.3 per cent., it occurred to the extent of 90–100 per cent. of the seed used in from 8 to 13 days, according to weather conditions. An attempt was made to determine the upper limit at which the maximum number of seed germinated. This was found to lie between 0.28 and 0.30 per cent. of copper. The figures in Table II are typical.

Table II.

March 1928		July 1928	
Copper added %	Percentage germination	Copper added %	Percentage germination
0.0	94	0.0	100
0.094	100	0.06	86
0.28	100	0.12	95
0.47	51	0.18	100
		0.24	95
		0.30	70

These results were duplicated several times and although the total germination may vary with conditions it is probable that above a concentration of 0.28 to 0.3 per cent. germination is definitely depressed.

That smaller concentrations have little permanent beneficial effect on germination is shown by the data in Table III, the results of which were obtained in July 1928.

Table III.

No.	Copper added %	Percentage germination		
		5 days	7 days	12 days
1	0.0	64	80	100
2	0.03	72	100	100
3	0.06	64	80	95
4	0.09	64	80	100
5	0.12	72	95	95
6	0.15	76	95	100

The figures in Table III suggest that in some cases germination has been stimulated. Unfortunately, however, the general effect on the seedlings is harmful. As soon as real growth is to be expected, the toxic effects of the copper manifest themselves. In general these consist of a darkening of the stems and cotyledons, and very pale leaves. An examination of the seedlings from the experiments quoted in Table III, 15 days after sowing, showed that there was little difference in Nos. 1, 2 and 3. The seedlings in No. 4 were dark in colour and not so healthy as the controls. Those in Nos. 5 and 6 were definitely stunted, the



cotyledons and stems being dark coloured and the young leaves pale. A few days later the seedlings in No. 2 were slightly taller than any of the others, but eventually none of those growing in the soil containing copper was as satisfactory as those in ordinary soil. In other words the stimulus to germination is more than nullified by the later toxic effects. Some of the seedlings from Nos. 1, 2 and 3 were potted up in ordinary soil and observations on them for the following three weeks revealed no differences. So the early benefit in germination is of no lasting value, even if the plants are potted up in new soil before the toxic effects of the copper appear.

Concurrently with these experiments with copper as sulphate some experiments were also made using Cheshunt Compound as source of copper. In all cases where any depressing effect was due to this mixture that effect was less marked than the effect due to the same concentration of copper added as the sulphate. The range of concentration over which Cheshunt Compound may be used is restricted as the ammonia in it is liable to scorch at concentrations very much above the normal strength, while even at low concentrations the ammonia is liable to stimulate the growth of young seedlings.

#### EXPERIMENTS WITH YOUNG TOMATO PLANTS.

The earlier experiments in this section were carried out in 1923 and 1924 and were arranged to compare the effects due to sulphate and those due to Cheshunt Compound. The compost consisted of a mixture of four parts of loam and one part of well rotted stable manure. During the period the light was never good, days were short and temperatures were too low for satisfactory growth. Under these conditions concentrations of the order of 0.2 per cent. of copper as sulphate caused the plants to be appreciably affected. When the copper was added as Cheshunt Compound, however, the plants were unaffected by considerably higher concentrations. Although growth was limited the plants affected by the copper showed the ill-effects within 3 days after planting. The foliage was exceptionally dark and some of the leaves were of a deep blue colour on the under sides. The roots were brown and apparently there was just sufficient activity to keep the plants alive but insufficient to enable growth to be made.

In the summer of 1924 attempts were made to determine the concentration of copper as Cheshunt Compound at which growth would be affected. If the mixture be used as directed by Bewley<sup>(1)</sup> the maximum amount applied to a young pot plant would not exceed 0.02 gm. of

## 434      *Effect of Copper Sulphate on Tomato Plants*

copper, per pot of 300 to 350 gm. of soil. At this concentration no ill-effects have been observed even under conditions very adverse to the well-being of the plant. On different occasions young plants were treated with amounts of the mixture containing up to 0.55 gm. of copper per pot of soil. Here again the effect of environmental conditions was apparent but in no case was any effect noticeable with amounts up to 0.21 gm. of copper. Mention may be made of a typical series in which 300 gm. of soil were used for each plant. Batches of sturdy plants were potted and they received respectively 0, 0.069, 0.137, 0.206, 0.274, 0.343, 0.411 gm. of copper as Cheshunt Compound. At the end of three weeks all the plants had made appreciable growth. No difference could be discerned in the appearance of the plants in the first four batches but those in the last three were not so tall although showing no untoward symptoms. That is, up to a concentration of about 0.07 per cent. copper as Cheshunt Compound is innocuous.

The question next arose as to how much copper would be likely to be present in the soil round a young plant where Cheshunt Compound had been used repeatedly for some years. This is only a remote possibility on a large nursery since the same soil is not likely to require treatment in consecutive years, but it may occur on small nurseries where steam sterilisation is not practised. As ordinarily recommended 2 pints of the solution would be applied to the soil round each plant in any one year. In 10 years this would amount to 2.5 gallons and if it be assumed that *none* of this be washed out in the course of ordinary cultivation the accumulation would amount to 1.370 gm. of copper. Assuming that this is distributed round the plant in 10 lb. of soil (the soil content of a large pot) the concentration of copper is of the order of 0.035 per cent. As already shown in the previous paragraph this concentration is quite harmless to young tomato plants.

### THE EFFECT OF COPPER ON MATURE TOMATO PLANTS.

The effect of copper in both of the forms mentioned has been examined on plants ranging from large plants growing in 6-inch pots to plants in 12-inch pots and carrying fruit. In the main the results have confirmed findings already mentioned regarding the comparative effects of the two forms of copper, that is to say, copper as sulphate is always more potent than copper in the form of Cheshunt Compound.

Under conditions prevailing during the winter months the effects of copper in small concentrations are so slight as to be almost indistinguishable from the effects of the unfavourable conditions. During the

summer months the poison symptoms are easily distinguished and are visible within 2 or 3 days. As an example may be quoted the results obtained from well-established plants of an average height of 11 inches potted up during July 1928. The soil contained copper as the sulphate in amounts varying from 0 to 0.604 per cent. In 3 days all the plants in soil with 0.07 per cent. or more were dark in colour and at the maximum concentration used most of them were shrivelled up and apparently dead. Within 5 days the symptoms of the surviving plants were accentuated and at concentrations of 0.15 per cent. and over the plants were all in a very unhealthy condition. It is interesting to note that 15 days later some of these plants were still alive. By that time most of the lower leaves were dead, the tips were very pale and scorched, and the stems showed long brown streaks. At concentrations up to 0.07 per cent. however, the only effect was one of slightly reduced growth.

These results confirmed those found in 1923 and 1924. In this latter case however, tests were also made with the copper as Cheshunt Compound. Up to concentrations of 0.25 per cent. of copper in this form none of the effects associated with the sulphate was observed. Above this concentration no reliable information could be obtained since the ammonia introduced in the mixture always scorched the foliage. Comparisons made at other times confirm the conclusion that copper as Cheshunt Compound is less harmful than the same concentration of copper as sulphate.

To examine the effects on mature plants large pots containing 12 lb. of soil were used and the plants were grown to a height of 4 feet or so, so that four trusses of fruit developed on each plant. Batches of soil were made up to contain copper as sulphate at concentrations of 0, 0.02, 0.04, 0.08, 0.12, 0.16 and 0.19 per cent. respectively. The plants were manured as often as was necessary and the fruit was picked as it ripened. Unfortunately none of the plants grew really well, irrespective of treatment, but it is noteworthy that even in the soil containing a concentration of 0.19 per cent. growth was made and fruit developed. The crop produced with 0, 0.02 and 0.04 per cent. was practically the same for all the plants. At the higher concentrations there was more variation in individual plants but the average per plant compared quite favourably with the control plants. As a matter of interest the seed in the fruit was saved. The different treatments appeared to be without any definite effect on the number of seed per fruit or on the germination of the seed. The subsequent seedlings however were all unsatisfactory for further growth, where the original plants had been treated with copper.

## TREATMENT OF POISONED SOIL.

The grower's most important concern is the treatment of soil which may have become poisoned, and the detection of the symptoms, which are more or less similar to those produced by most poisons. In seedlings the stems become blue or violet in colour and the cotyledons a dark green. The rough leaves are dark green on making their appearance but soon become a pale yellow, and the veins show in bold relief. These symptoms are accompanied by stunted growth and sometimes by distortion.

In young pot plants severe poisoning is accompanied by shrivelling of the lower leaves, brown streaks on the stems, and the leaves at the tips become yellow on the upper surface and often blue or violet on the under side. These plants do not necessarily die, but little or no apparent growth is made and when the lower leaves have fallen off the plants consist of vertical stems each surmounted by a tuft of stunted leaves.

With large plants the effects are not so definite and even with comparatively large amounts of copper no striking differences are noticeable between treated and control plants.

*Washing with water.*

When the concentration of copper in the soil is low much benefit is derived from heavy watering. When the concentration is so high as to cause immediate (*i.e.* within 2 days) injury no amount of water appears to remove the copper completely. For instance, in one series of experiments, some pots of soil which was highly toxic were watered daily for three weeks so that water flowed freely through the drainage holes on each occasion. At the end of this time the leachings, after concentration, showed none of the usual qualitative tests for copper. On planting in this soil the symptoms did not appear immediately but were quite definite at the end of eight days.

*The effect of lime.*

Hydrated lime has been found to give satisfactory results when applied to poisoned soil. In mild cases of poisoning a light dressing at the soil surface is usually sufficient to encourage normal growth. With more severe cases it is necessary to mix the lime with the soil and water the whole heavily, and when the mixing is really thorough the amount of copper which is rendered innocuous is surprising.

*The effect of manures.*

Stable manure, sulphate of ammonia, sulphate of potash and basic slag have all been found of some benefit. None of these, however, is as effective as hydrated lime. Of the four, application of sulphate of ammonia followed by watering is the most satisfactory.

*The effect of soiling.*

On many occasions plants showing the typical symptoms have made good recovery when one or two inches of "clean" soil have been added round the stems. Even in seed boxes where there has been little room for the addition of such soil seedlings have made excellent recovery after this treatment. This confirms the view that copper, although causing the plant very severe injury, often does so without actually killing it, and that the plant can live for appreciable periods in the presence of a toxic concentration. When more favourable root conditions prevail, as when new soil is added, new roots are formed and new growth is made.

## SUMMARY AND CONCLUSIONS.

An examination has been made of the effects of copper on tomato plants at various stages of growth.

No benefit appears likely to accrue from the addition of copper sulphate to tomato soils. The rate of germination may be stimulated but the total germination is unaffected and the subsequent growth of the seedlings is not satisfactory.

The toxicity of copper is greater when used as sulphate than when added in the form of Cheshunt Compound.

Cheshunt Compound, introduced as an emergency measure for the control of "damping off" of tomato seedlings and foot rot, is harmless when used as originally directed.

When soil is poisoned the addition of new soil round the stems of plants and the application of hydrated lime are beneficial.

The addition of small amounts of copper sulphate to soil in which mature plants are grown is without effect on the number of seed per fruit and on the ability of the seed to germinate, but the subsequent seedlings are unsatisfactory from the commercial point of view.

This work was carried out at the suggestion of Dr W. F. Bewley, to whom the author tenders his best thanks.

## REFERENCE.

- (1) BEWLEY (1921). Experimental and Research Station, Cheshunt. *Ann. Repts.* vii, 38.

*(Received January 19th, 1929.)*

## SOME PROPERTIES OF THE CELL-WALL OF COTTON-HAIRS

By N. W. BARRITT, M.A.

(*Late Senior Botanist, Cotton Research Board, Egypt.*)

(With 3 Text-figures.)

THE structure of the cell-wall of the cotton-hair and its behaviour under the action of various reagents have been the subject of investigation by numerous workers. Denham<sup>(1)</sup> and Clegg and Harland<sup>(2)</sup> have drawn attention to its porosity, whilst Calvert and Summers<sup>(3)</sup>, and Coward and Spencer<sup>(4)</sup>, have shown that the dried hair absorbs caustic soda solution until it assumes the same diameter that it has as a living cell inside the boll.

The relation of this porosity of the cell-wall to nutrition has been discussed by the present writer elsewhere<sup>(5)</sup>, and subsequent investigations of the swelling of the cell-wall, before collapse occurs, have revealed unsuspected features correlating the somewhat difficult problems of hair weight, diameter and intrinsic strength.

### METHOD.

The apparatus used in these investigations was a Leitz microscope projection apparatus with three lens substage condenser and centring mount, oil immersion lens, four eye-piece, and reflecting prism adjusted to give a magnification of 1250. With critical illumination and working within a radius of three inches from the centre of the field of projection, diameter measurements of 300 cotton-hairs can be made in one hour with an accuracy of  $\pm 2$  per cent. without any strain or fatigue to the eyes.

#### 1. STABILITY OF THE CELL-WALL.

Diameter measurements were made on a tuft of hairs from an unripe boll of Ashmuni cotton 35 days old, mounted in tap water, 5 per cent. salt solution, saturated brine, 5 per cent. formalin and after immersion in boiling water. The same tuft of hairs was used for the various treatments, and all observations were made at the centre of the hair length. This is

facilitated by taking a tuft of hairs (say 150) attached to a portion of the seed coat. They are straightened out by combing under water and when mounted on the slide, the piece of seed coat is used as a stop for one edge of the coverslip from which to measure the centre of the hairs. The results are given in Table I.

Table I.

Treatment	Mean diameter in 1/1250 mm.
Tap water	27.3
5 % salt solution	27.4
Saturated brine	27.3
5 % formalin, 1st day	27.6
5 % formalin, 2nd day	27.6
After boiling water	27.1
Dried and mounted in water	21.8
Swollen in 18 % NaOH	26.9

From these results it appears that the diameter of the hair is unaffected by salt solution, formalin or boiling water. It is therefore concluded that the diameter of the cotton-hair is not dependent on osmosis or the vital activity of the cell, and that collapse of the hair on maturation is entirely a physical process dependent on dehydration of the cell-wall.

In the above instance, the original diameter of the hair was almost restored by immersing the collapsed hair in 18 per cent. caustic soda, thus supporting the theory that the cuticle determines the diameter of both the living and the "swollen" hair.

Fig. 1 is a reproduction of a photograph of living hairs mounted in water. One of the hairs shows the effect of partial removal of the primary wall and cuticle by friction. It will be seen that removal of the primary wall results in an expansion of the hair-cell, from which it must be concluded that the cuticle of the living hair is under tension.



Fig. 1.

## 2. COMPARISON OF DIFFERENT STRAINS OF COTTON UNDER THE ACTION OF CAUSTIC SODA SOLUTION.

Previous workers have maintained that the limiting action of the cuticle on cell diameter applies to all cottons alike. Although Summers's results show differences between fine and coarse cottons, he attributed them to experimental error. The present writer found much greater differences than those of Calvert and Summers, and such as to be unaccountable by error. This could be explained by the fact that their material was grown in England under artificial conditions, and only one sample could be described as a fine cotton.

By making the measurements in water and caustic soda solution on the same tuft of hairs at the same portion of their length, the possibility of sampling error is avoided and the results have an accuracy of  $\pm 2$  per cent.

The results obtained for 20 strains of cotton are given in Table II, arranged in order of "living" diameter.

Table II.

Sample	Diameter measurements in 1/1250 mm.		Percentage increase in diameter
	In water	In 18 % NaOH solution	
1	19.1	21.8	14.0
2	19.6	22.8	16.2
3	20.6	23.3	13.2
4	21.0	23.8	13.3
5	21.1	24.8	17.6
6	21.7	24.2	11.5
7	22.4	25.1	12.0
8	22.8	24.6	7.9
9	23.0	24.5	6.5
10	23.1	24.7	7.0
11	23.4	25.4	8.5
12	23.7	25.3	6.7
13	25.2	27.0	7.1
14	26.0	24.8	4.6
15	26.2	26.6	1.5
16	27.3	26.9	1.4
17	28.2	28.3	0.3
18	28.2	27.6	2.1
19	32.1	32.3	0.6
20	33.5	32.6	2.8

The results in the third and fourth columns are plotted in a curve (Fig. 2,) which shows that the increase in diameter on swelling the cell-wall with caustic soda solution varies inversely as the diameter of



the uncollapsed hair; and that when the diameter exceeds  $26/1250$  mm., there is no increase in diameter on swelling the cell-wall, but sometimes an apparent decrease. This unexpected decrease in diameter of the coarser hairs may be accounted for by a change in shape of the cross-section from an ellipse to a circle (see Fig. 3).

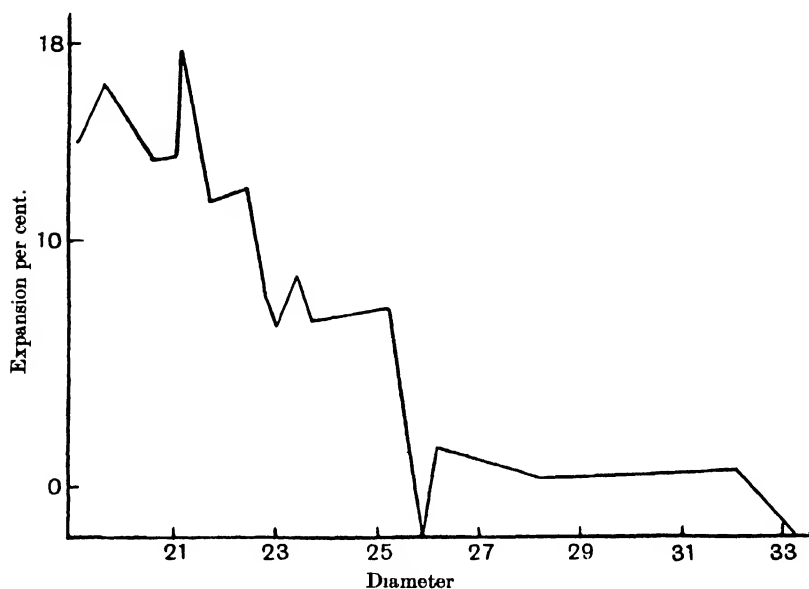


Fig. 2.

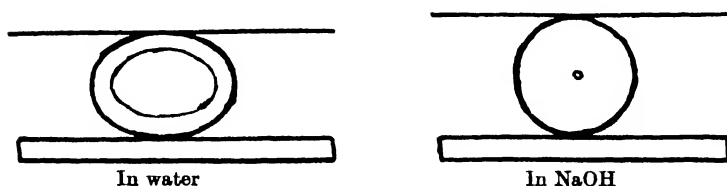


Fig. 3.

Thin-walled hairs of large diameter and a correspondingly large lumen would tend to assume an elliptical shape between the slide and coverslip, but, on swelling the cell-wall with caustic soda, the increased internal pressure would tend to "inflate" the hair into a more cylindrical tube.

This source of error in the measurement of the living hairs, due to the unavoidable presence of thin-walled hairs, would enter into all the

## 442 *Some Properties of the Cell-Wall of Cotton-Hairs*

determinations irrespective of the variety of cotton, so that the expansions recorded for the finer cottons may be actually less than their true values. The varying amounts of this error would account for the irregularities in the correlation curve.

This increased expansion of the finer cottons is very remarkable, especially as the four cottons with the greatest expansion represent strains of Sakel remarkable for their superior spinning tests. No. 5, which gave the highest percentage expansion (17·6), possesses the highest intrinsic strength of any cotton grown in Egypt<sup>(6)</sup>, whilst the four cottons at the bottom of the curve comprise one American, two coarse strains of Ashmuni and a sample of native brown cotton of no commercial value.

That there is some relation between original diameter and intrinsic strength is shown by the results in Table III for six different strains of cotton.

Table III.

Diameter of living hair in water	Intrinsic strength
19·1	283
21·0	303
22·4	260
22·8	267
28·2	223
33·5	207

Not only is this true for different strains of cotton, but it also holds good for cotton of the same strain. A sample of Sakel cotton was divided into bundles of hairs of different weights per cm., and the hair strength determined by means of the magazine hair tester.

The results are shown in Table IV.

Table IV.

No. of hairs in bundle	Hair weight per cm.	Hair strength	Intrinsic strength
75	13·3	4·0	300
81	13·7	4·23	308
76	17·0	4·43	260
83	17·2	4·7	273

It is reasonable to assume that, in cotton from the same plants, the hair weight per cm. is proportional to diameter. The above results, though few in number, are free from any sampling error, and show a definite relation between diameter and intrinsic strength.

## DISCUSSION.

The familiar "beading" of cotton hairs with a damaged cuticle on treatment with caustic soda is conclusive evidence that the cuticle determines the final diameter of the swollen cell-wall of all types of cotton.

To account for the increased diameter on swelling of fine cottons, we must suppose the cuticle to be more extensible than that of coarse cottons, and that it is probably subject to a greater pressure from the swollen cell-wall. The higher intrinsic strength of the cell-wall of fine cottons suggests a different molecular aggregation or polymerisation of the cellulose molecules, which would be associated with a greater absorption and swelling power in caustic soda solution.

The higher intrinsic strength of the finer hairs over the coarser hairs of the same plants is indeed remarkable, and suggests that the inherent properties of the cellulose (or degree of polymerisation) are in some way connected with the dimensions of the living hair. How this can occur is at present a subject only for speculation. The existence of internal pressure in the cell-wall of the living hairs, and the greater surface area for nutrition possessed by hair cells of small diameter, may well be the basis of all the complex factors determining quality in cotton.

## SUMMARY.

(1) The cell-wall of the living cotton-hair is under tension and its diameter is unaffected by osmotic pressure or the death of the cell.

(2) Increase of diameter of the uncollapsed hair by swelling in caustic soda varies inversely as the diameter of the hair in the case of fine cottons, and does not occur with coarse cottons.

(3) The diameter of the uncollapsed cotton-hair varies inversely as the intrinsic strength of the collapsed hair.

I have to thank Dr R. H. Pickard, F.R.S., and his colleagues Miss Calvert, M.Sc., and Miss Clegg, M.Sc., of the Shirley Institute, Manchester, for their very helpful criticisms.

## REFERENCES.

- (1) DENHAM (1923). The structure of the Cotton Hair. *Journ. Text. Inst.* xiv, No. 3, T. 86.
- (2) HARLAND, S. and CLEGG, G. (1923). Measurable Characters of Raw Cotton. *Ibid.* T. 489.
- (3) SUMMERS, F. and CALVERT, M. A. (1926). *Memoirs of Shirley Institute*, iv, No. 5.
- (4) COWARD and SPENCER (1923). *Journ. Text. Inst.* xiv, T. 32.
- (5) BARRITT, N. W. (1928). *Report of British Association and Annals of Botany* (1929), XLIII, No. CLXXI, pp. 1-7.
- (6) — (1929). *Journ. Text. Inst.* xx, No. 4, T. 70.

(Received December 10th, 1928.)

# THE PRODUCTION OF ETHYL ALCOHOL AND ACETALDEHYDE BY APPLES IN RELATION TO THE INJURIES OCCURRING IN STORAGE

## PART I. INJURIES TO APPLES OCCURRING IN THE ABSENCE OF OXYGEN AND IN CERTAIN MIXTURES OF CARBON DIOXIDE AND OXYGEN

By MEIRION THOMAS, M.A.

*(Department of Botany, Armstrong College, Newcastle-upon-Tyne, formerly of the Low Temperature Research Station, Cambridge.)*

(With Plate XX.)

### CONTENTS.

	PAGE
INTRODUCTION . . . . .	444
1. INJURY TO APPLES FOLLOWING ANAEROBIC ZYMESIS . . . . .	447
(i) Invasive alcohol poisoning . . . . .	447
(ii) Diagnosis by chemical analysis of invasive alcohol poisoning . . . . .	448
2. INJURIES TO APPLES FOLLOWING CO <sub>2</sub> -ZYMESIS . . . . .	449
(i) Internal aldehyde poisoning and invasive aldehyde poisoning . . . . .	449
(ii) Diagnosis by chemical analysis of internal aldehyde poisoning and invasive aldehyde poisoning . . . . .	452
(iii) Aldehyde poisoning in relation to the physiological state of apples, and to the concentration of carbon dioxide and oxygen. Gas storage . . . . .	452
(iv) Brown-heart as a form of injury which is possibly different from internal aldehyde poisoning . . . . .	454
SUMMARY AND CONCLUSIONS . . . . .	454
ACKNOWLEDGMENTS . . . . .	456
REFERENCES . . . . .	456

### INTRODUCTION.

IN recent years problems of apple storage and the transport of apples from overseas have been engaging the attention of many workers in this and other countries. Some of the work is directed at providing stores in which home-grown apples may be kept in a marketable state for longer periods than in the past. Other work is proceeding on the conditions which prevail in the holds of ships carrying apples for long

distances, and often, indeed, across the equator in the summer. In general it may be said that the aim of all the work is to arrange storage conditions so that the risk of fungal and bacterial rot is minimised, and so that the inherent processes of senescence of apples can be retarded, or accelerated, at will.

The storage of home-grown apples and of apples in ships' holds in freely circulating air at low temperatures has already proved its worth, although, as we shall now see, it is not wholly without danger. In order to prevent freezing injury, the temperature should never be allowed to fall below 0° C., but even at temperatures ranging from 1° C. to 5° C. it has been found that certain varieties of apples may be injured more quickly than when they are stored at higher temperatures, and that these injuries are not brought about by micro-organisms. Names, such as "deep- or soft-scald," and "internal-breakdown," have been given to those physiological diseases which seem to be more often encountered at low temperatures(2, 4). These diseases will be considered in a later paper.

The storage of fruit in atmospheres other than air has often been considered. In gas storage trials carried out by Kidd and West(5) certain varieties of apples have been stored in an atmosphere of approximately 10 per cent. carbon dioxide + 10 per cent. oxygen + 80 per cent. nitrogen. The increase in the concentration of carbon dioxide in conjunction with the decrease in that of oxygen retards metabolic activity and so delays the ripening of the fruit. These workers have found that a temperature of 8–10° C. gives the most satisfactory results. No injurious effects have been noticed under these abnormal conditions so long as the composition of the gas mixture was rigidly controlled. If however, as a result of the respiration of the apples, the concentration of carbon dioxide increased even to 13–14 per cent., then, as Kidd and West have conclusively shown(3), a physiological disease incident in the interior, which they have called brown-heart, was liable to occur, provided that oxygen was still present.

Such harmful gas conditions as those described in the last paragraph may arise from the respiration of apples in air stores which are insufficiently ventilated, as indeed has happened in certain types of ships' holds carrying fruit, and, as a result, brown-heart has occurred on a large scale(3). Further, if a gas store or an unventilated air store is left without control, much if not all of the oxygen may be used up by the respiration of the apples which are then, as has long been known, sooner or later killed.

It has been recognised for some years that although the flesh tissue of apples appears to the eye to be homogeneous, it may behave as a heterogeneous population of cells when stored under adverse conditions, in that usually the cells are not all injured simultaneously. Indeed, up to the present, the physiological diseases of apples have been distinguished from one another mainly by the different incidence of injuries and the variations shown in their subsequent extension<sup>1</sup>. These differences and variations are implicit in the definite names, such as superficial-scald, deep- or soft-scald, internal-breakdown, brown-heart, which have been given to these diseases. Although these terms have proved very useful, they are not wholly satisfactory as they do not even adumbrate the causes of the diseases. Moreover, the same injurious effects may be produced under quite different storage conditions: for example, injuries incident in the epidermis and progressing inwards, often spoken of as deep- or soft-scald, have been reported as occurring in air stores at low temperatures (2, 3), in anaerobic stores at all temperatures (2, 3), and in stores containing certain mixtures of carbon dioxide and oxygen (1, 6). It is thought that it would be preferable in describing physiological diseases to use terms which both indicate the nature of the browning manifestations, and suggest some distinctive internal physical or chemical circumstance which either causes or can be correlated with the disorganisation of cells which always precedes browning.

A great deal of difficult work must be done before such a nomenclature can come into being, as a wide knowledge of the biophysical and biochemical changes, which occur under different conditions of storage, would be required. With this knowledge it is considered that it might be possible, after diagnosing a physiological disease by physical or chemical tests and by its appearance, to say what environmental conditions had caused the disease. Further, the chance of preventing physiological diseases will probably be greater when the internal phenomena associated with them are more fully understood. It was with a view to investigating the biochemical phenomena associated with these diseases that the author commenced this work under the Food Investigation Board in 1922. The experiments to be discussed under the title of this paper were planned to determine whether the injuries occurring in storage were preceded, accompanied or followed by zymasis. During this metabolic disorder the concentration of ethyl alcohol and acetalde-

<sup>1</sup> It must be remembered that the apple is composed of oxidase tissue, so that injury is always accompanied by browning if oxygen is present to complete the catechol-oxidase system (7).

hyde in cells progressively increases from the small amounts of these substances—ethyl alcohol 0.006 per cent.<sup>1</sup>, acetaldehyde 0.0005 per cent.<sup>1</sup>—found in freshly gathered apples. The author has, in an earlier paper<sup>(9)</sup>, described the methods of analysis which he uses in following zymasis. In this communication the injuries occurring in apples in certain gas atmospheres, other than air, will be considered.

In most of the work Newton Wonder apples were used, but similar experiments on other apples have been made, and so far have given results in general agreement with those which will now be described. It is realised, however, that many differences in detail may be found in further work on other varieties, and that a given variety may be found to behave differently according to its physiological state.

#### SECTION 1. INJURY TO APPLES FOLLOWING ANAEROBIC ZYMASIS.

(i) *Invasive alcohol poisoning.* It has been reported elsewhere<sup>(8)</sup> that whereas zymasis does not occur in healthy apples stored in CO<sub>2</sub>-free atmospheres containing more than 5 per cent. oxygen, it commences when the external oxygen concentration falls below some value between 1 and 5 per cent., and is of a kind similar to that which takes place in the complete absence of oxygen. Such anaerobic zymasis continues so long as some of the cells remain alive, and is characterised by a relatively great accumulation of ethyl alcohol, and a small accumulation of acetaldehyde within the tissues. From Section 2, Table II, it will be seen that in apples which have been stored in 100 per cent. nitrogen, the ratio ethyl alcohol/acetaldehyde is usually > 50/1. Injuries to, and death of, the cells of an apple generally accompany these metabolic changes if anaerobic conditions are maintained for long enough. Progressive stages of these injurious effects are shown on Plate XX, figs. 1 *a* and 1 *b*. It should be noticed that injury is usually incident in the epidermal cells, and progresses inwards, until finally the whole apple may be killed. The photographs were taken some time after removal from the anaerobic conditions to air, so the injured and killed tissue had turned brown. Acetaldehyde ceases to accumulate in apples some time before injury is apparent and indeed before the rate of ethyl alcohol formation by anaerobic zymasis is retarded to any extent<sup>(8)</sup>. Further, since the data which will be presented in Section 2 indicate that acetaldehyde in concentrations less than 0.01 per cent. is not toxic to apple cells, and since the amount of this substance has never been found to exceed 0.006 per

<sup>1</sup> Results are expressed throughout in terms of gms. acetaldehyde or ethyl alcohol found in 100 gms. fresh weight of the tissue of an apple or a sample of apples.

## 448 *Ethyl Alcohol and Acetaldehyde produced by Apples*

cent. after anaerobic zymasis, there is no evident connection between acetaldehyde and the injuries occurring under anaerobic conditions. The results given in Table I, however, suggest that there may be a relation between such injuries and the concentration of ethyl alcohol found by analysis in apples removed from anaerobic stores.

Table I.

*On the relation between ethyl alcohol production and invasive alcohol poisoning in anaerobic storage.*

Temperature of storage chamber	No. of days in anaerobic store before invasive alcohol poisoning noticed	Percentage of ethyl alcohol in apples showing invasive alcohol poisoning
22° C.	5	0.368
15° C.	9-13	0.396-0.504
1° C.	38	0.389

Although the data are limited in number, we are probably justified in concluding from this table that an accumulation of ethyl alcohol of the order 0.3 per cent. will, at all temperatures, precede the commencement of injury to apple tissue under anaerobic conditions, and so of the appearance of browning effects when such injured apples are subsequently exposed to air. Since we shall in the next section describe injuries incident at the surface of apples and progressing inwards which are brought about by quite different conditions, it is proposed to speak of the type of injury following a deficiency of oxygen alone as "invasive alcohol poisoning." It is not asserted that ethyl alcohol is the sole cause of these injuries<sup>1</sup>, but it certainly appears to be the best index of the general state of poisoning which follows anaerobic zymasis.

(ii) *Diagnosis by chemical analysis of invasive alcohol poisoning.* It is probable that invasive alcohol poisoning can be diagnosed as such by chemical analysis of apples, because concentrations of ethyl alcohol of the order 0.3 per cent. have never been found in apples excepting after anaerobic storage. We shall see in the next section that it may be further distinguished from the invasive injuries resulting from an excess of carbon dioxide in the presence of oxygen, by the value of the ethyl alcohol/acetaldehyde ratio. Since neither ethyl alcohol nor acetaldehyde is oxidised by apple cells, and since these substances are not lost by evaporation with any considerable degree of rapidity<sup>(8)</sup>, it is permissible to delay the analyses of apples suspected to be suffering from invasive alcohol poisoning for several days after removal from the conditions

<sup>1</sup> Attempts to produce differential browning in halved apples placed in the vapour of ethyl alcohol failed. The surfaces of the apples, if they were quite healthy at the start, browned uniformly.



which induced zymasis, provided that micro-organisms have not subsequently changed the products of metabolism. This is an important point, for it follows that apples arriving from overseas in a damaged condition may be held in store for a short time before being analysed, without necessarily invalidating the results of the analysis. Further, all the results so far obtained indicate that anaerobic zymasis occurs at the same rate and to the same extent in all parts of the flesh tissue of a single apple<sup>1</sup>. Hence, analysis of the remaining sound parts of an apple, suspected to be suffering from invasive alcohol poisoning, will be sufficient for diagnostic purposes, and will certainly, in a delayed analysis, give more trustworthy results than those which would be obtained from damaged parts in which micro-organisms will probably have been active.

## SECTION 2. INJURIES TO APPLES FOLLOWING CO<sub>2</sub>-ZYMESIS.

(i) *Internal aldehyde poisoning and invasive aldehyde poisoning.* A full account has been given elsewhere<sup>(8)</sup> of the discovery that carbon dioxide above certain concentrations, even in the presence of abundant oxygen, may cause the respiratory metabolism of glucose by apple cells to be changed to a zymasic type. Although the end products of this CO<sub>2</sub>-zymasis are the same as those of anaerobic zymasis, it has been found that during CO<sub>2</sub>-zymasis more acetaldehyde and correspondingly less ethyl alcohol accumulates in the cells. Indeed, during and after CO<sub>2</sub>-zymasis the ratio ethyl alcohol/acetaldehyde is approximately 2/1—in striking contrast to the ratio 50/1 already recorded as characterising anaerobic zymasis. The continuous accumulation of acetaldehyde during CO<sub>2</sub>-zymasis, which leads to the differences which have just been noted, is correlated with the presence of oxygen in the environment.

The toxicity of acetaldehyde is greater than that of ethyl alcohol, so it is not surprising that gas mixtures containing both carbon dioxide and oxygen may prove more harmful to apples than anaerobic conditions whether imposed by using pure carbon dioxide, pure nitrogen, or any mixture of these two gases. After CO<sub>2</sub>-zymasis, injury which is accompanied by browning, since oxygen is present, is usually first incident in the interior (see Plate XX, fig. 2 *a* and Table II (A)), and not until later does invasive browning from the epidermal cells inwards occur (see Plate XX, fig. 2 *b* and Table II (B)); finally the whole apple may be killed (see Plate XX, fig. 2 *c* and Table II (D)).

<sup>1</sup> It seems from this, that the incidence of invasive alcohol poisoning in the epidermal cells of apples must be ascribed to the greater susceptibility of these cells to the adverse consequences of anaerobic conditions. Incidence of alcohol poisoning in the interior, to give "internal alcohol poisoning," is of course possible but has not yet been encountered.

## 450 *Ethyl Alcohol and Acetaldehyde produced by Apples*

The results presented in Table II were obtained from experiments in which apples were subjected to chemical analysis after storage at two different temperatures for different time periods in gas mixtures of carbon dioxide and oxygen, or under anaerobic conditions. These results serve for the comparison of the toxic effects resulting from CO<sub>2</sub>-zymasis with those following anaerobic zymasis, whether induced by pure nitrogen or pure carbon dioxide.

Table II.

*Comparison of toxicity of CO<sub>2</sub>-zymasis and anaerobic zymasis.*

Temperature 1° C.				
Days in chamber	Gas mixture of 50 % carbon dioxide + 50 % oxygen in storage chamber		100 % nitrogen in storage chamber	
	Ethyl alcohol %	Acetaldehyde %	Ethyl alcohol %	Acetaldehyde %
3	—	0.01	—	—
7	0.05	0.02 (A)	—	—
15	0.075 (B)	0.03 (B)	0.17	0.0035
23	0.08 (D)	0.04 (C)	0.25	0.004
38	0.08 (D)	0.04 (D)	0.39 (C)	0.004 (C)

Temperature 15° C.			
Days in storage chamber	Gas mixture of 70 % carbon dioxide + 30 % oxygen in storage chamber.		100 % carbon dioxide in storage chamber.
	Acetaldehyde %		Acetaldehyde %
3	0.005		—
5	0.010 (A)		0.005
11	0.030 (B)		0.006
20	— (D)		0.006 (C)

(A) Visible browning in the interior. "Internal aldehyde poisoning" (see Plate XX, fig. 2 a).

(B) Visible browning incident in epidermis and progresses inwards. "Invasive aldehyde poisoning" (see Plate XX, fig. 2 b).

(C) Injury incident in epidermis and progresses inwards. Injured tissue browns on subsequent exposure to oxygen. "Invasive alcohol poisoning" (see Plate XX, fig. 1 a).

(D) Apples completely killed (see Plate XX, figs. 1 b and 2 c).

It will be seen that at both temperatures the apples used were more quickly injured and killed in gas mixtures of carbon dioxide and oxygen than when under anaerobic conditions. Further, at 1° C. after CO<sub>2</sub>-zymasis apples may be completely killed when they contain only 0.08 per cent. ethyl alcohol, whereas apples containing 0.25 per cent. ethyl alcohol after anaerobic zymasis generally show little or no sign of

injury when placed subsequently in air. So it seems to be very significant that when injury is first seen after  $\text{CO}_2$ -zymasis at both temperatures (injury A), there is present in the tissues a greater concentration of acetaldehyde (0.01–0.02 per cent.) than has as yet been found to accumulate by anaerobic zymasis. Not all apples show this internal injury after  $\text{CO}_2$ -zymasis, and it is important to notice that it has never been encountered after anaerobic zymasis; two findings, which are in accord with the conclusions of Kidd and West, are that “brown-heart” only occurs in apples stored in chambers containing carbon dioxide and oxygen, and that the susceptibility of apples to this disease varies greatly (3). A consideration of the invasive injuries (injuries B and C), which occur after both these unusual respiratory processes have been in progress for some time, leaves little doubt that at both  $1^\circ\text{C}$ . and  $15^\circ\text{C}$ . their earlier appearance and more rapid spread in gas mixtures of carbon dioxide and oxygen may be correlated with the accumulation, under these conditions, of an inducing concentration of 0.03–0.04 per cent. acetaldehyde in the tissues, many days before the products of anaerobic zymasis have reached a toxic limit in apples placed under anaerobic conditions for the same time periods and at the same temperatures. Indeed so poisonous is acetaldehyde that apples are, as a rule, completely killed when 0.04 per cent. has accumulated in them.

It seems probable that both the internal and invasive brownings, which occur after  $\text{CO}_2$ -zymasis in apples stored in carbon dioxide and oxygen, are actually caused by the acetaldehyde which is produced. Support is given to this opinion by the results of experiments on apples which were first halved by transverse cuts, and then placed in covered desiccators at  $16$ – $18^\circ\text{C}$ . over freely evaporating acetaldehyde: after 24 hours internal flesh browning was seen in some of the apples (see Plate XX, fig. 3 *a*), although during this period very little carbon dioxide had accumulated in the desiccators; and, later, invasive browning occurred (see Plate XX, figs. 3 *b* and 3 *c*). Clearly the localisation and succession of injuries in halved apples in pure acetaldehyde vapour is the same as when this substance is formed within the cells by  $\text{CO}_2$ -zymasis. Hence it is thought that we may rightly speak of the injuries occurring in apples during  $\text{CO}_2$ -zymasis as “internal aldehyde poisoning” (Table II (A)) and “invasive aldehyde poisoning” (Table II (B)) (cf. remarks in Section 1 on “invasive alcohol poisoning”).

Evidently the differential browning produced by acetaldehyde vapour is due to the different susceptibilities of the cells of apples to this substance. Suggestions, supported by analyses of the internal

atmosphere of apples, have been made that the deep-seated and localised incidence of the injury called brown-heart may be due to the danger limit of carbon dioxide being reached earlier in certain localities within this bulky fruit (3). This explanation, however, will not hold for all cases of internal aldehyde poisoning after  $\text{CO}_2$ -zymasis, as halved apples placed in 50 per cent. carbon dioxide + 50 per cent. oxygen may show the same sequence of injuries as are seen in whole apples after  $\text{CO}_2$ -zymasis (see Plate XX, figs. 2 a, 2 b and 2 c). It seems to be better to think of the differential browning following  $\text{CO}_2$ -zymasis as due to the different susceptibilities of the cells of apples to the acetaldehyde which is produced.

(ii) *Diagnosis by chemical analysis of internal aldehyde poisoning and invasive aldehyde poisoning.* It may in future be possible to diagnose by chemical analysis internal aldehyde poisoning in apples brought to the laboratory for examination, and which on cutting show internal browning effects, since values of acetaldehyde of the order 0.01 per cent. or more will only be given if  $\text{CO}_2$ -zymasis has occurred. Certainly, as the figures assembled in Table III show, it should always be possible to distinguish invasive aldehyde poisoning from invasive alcohol poisoning.

Table III.

*Distinction between invasive aldehyde poisoning and invasive alcohol poisoning by chemical analysis.*

	After invasive aldehyde poisoning appears	After invasive alcohol poisoning appears
Concentration of ethyl alcohol in apples	Usually < 0.1 %	Usually > 0.3 %
Concentration of acetaldehyde in apples	Of the order 0.03 %	Usually < 0.006 %
Ethyl alcohol/acetaldehyde	Of the order 2/1	> 50/1

The numbers relating to invasive alcohol poisoning seem to be independent of the gas in which anaerobic zymasis occurs: for example, they hold good for apples kept in pure nitrogen or pure carbon dioxide. The numbers relating to invasive aldehyde poisoning have only been obtained in gas mixtures of carbon dioxide and oxygen, and they appear to be quite independent of the concentration of carbon dioxide, provided this is sufficient, in the first place, to induce zymasis (8).

(iii) *Aldehyde poisoning in relation to the physiological state of apples, and to the concentration of carbon dioxide and oxygen. Gas storage.* In these experiments  $\text{CO}_2$ -zymasis occurred practically at once in all apples placed in gas mixtures containing more than 50 per cent. carbon dioxide,

provided that at least 5 per cent. oxygen was present<sup>1</sup>, and under these conditions the apples were poisoned very quickly by the acetaldehyde which was produced. Considerable variation of resistance towards concentrations of carbon dioxide which were less than 30 per cent. was, however, shown<sup>2</sup>; some apples underwent CO<sub>2</sub>-zymasis and showed the injurious after-effects of this more rapidly, and others more slowly<sup>(8)</sup>. Further evidence of the variation of the resistance of apples towards carbon dioxide has been given by Kidd and West in their report on brown-heart<sup>(3)</sup>; and also by workers in the U.S.A. on the development of "disagreeable alcoholic flavours" and of tissue browning in apples placed in gas mixtures containing carbon dioxide and oxygen<sup>(6)</sup>. Possibly the cell metabolism of apples is disordered by progressively lower concentrations of carbon dioxide as the apples become more senescent. If this is so, then the temperature of storage and the concentration of oxygen in the store will both be of great importance in relation to these phenomena, for there can be little doubt that the rate of the march of senescence can be accelerated, within limits, by raising the temperature of the store, or by increasing the oxygen concentration in it. In this connection the following results of analyses are significant:

Exp. (a). Apples gave no products of zymasis after "gas storage" for 6 months at 8° C. in a gas mixture containing 10 per cent. carbon dioxide, 10 per cent. oxygen and 80 per cent. nitrogen (see Introduction).

Exp. (b). A gas stream containing 12 per cent. carbon dioxide and 88 per cent. oxygen was passed over apples, which had previously been stored in air at 1° C. for 6 months, placed in desiccators at 15° C. Zymasis occurred after 21 days, and injurious effects were seen later. It is suggested that during the 21 days the senescence processes in the apples were so hastened by the high concentration of oxygen acting in conjunction with the increased temperature, that the resistance of the apples to carbon dioxide was rapidly lowered, and finally zymasis occurred.

Although Exp. (b) is not a strict control for Exp. (a), nevertheless these experiments when considered together indicate that subnormal concentrations of oxygen are effective in maintaining the resistance of

<sup>1</sup> With concentrations of oxygen <5 per cent. the average metabolism of the whole apple is expressed by numbers intermediate between those characterising anaerobic zymasis and CO<sub>2</sub>-zymasis<sup>(8)</sup>.

<sup>2</sup> No experiments have as yet been carried out with concentrations of carbon dioxide between 30 per cent. and 50 per cent.

apples to moderate strengths of carbon dioxide; hence, the efficiency of gas storage as practised by Kidd and West (see Introduction).

We conclude that there are some conjunctions of carbon dioxide and oxygen in which apples may be kept without zymasis or injury occurring, but that there are others in which all apples will show zymasis followed by aldehyde poisoning: in conjunctions intermediate between these, apples will respond according to their physiological states.

(iv) *Brown-heart as a form of injury which is possibly different from internal aldehyde poisoning.* In the course of their preliminary gas storage trials with apples, Kidd and West noticed that an injury, which they have called "brown-heart," sometimes occurred when the concentration of carbon dioxide in the storage atmosphere exceeded 13 per cent.(3). After the discovery of the phenomenon of  $\text{CO}_2$ -zymasis, it was naturally attractive to think that brown-heart injury is preceded by and caused by the formation, possibly locally, of acetaldehyde in apples under such conditions. Analyses of apples which had suffered during gas storage trials from brown-heart gave no evidence, however, that zymasis had occurred during the storage period. Since the injured tissue was shrivelled into a dry mass when the analyses were made, it is conceivable that zymasis had occurred, possibly locally, a considerable time previously, and that the products of zymasis had in the interval disappeared from the apples by diffusion or through some other cause. Further, Australian apples, suffering from what seemed to be typical brown-heart, have been analysed when the brown injured tissue was still soft and very moist. Small amounts of ethyl alcohol (0.09 per cent.) and acetaldehyde (0.004 per cent.) were recovered. Since, as will be shown in a later paper, some form of zymasis follows many different forms of injury to apple tissue, it is difficult at present to say whether the alcohol and aldehyde found in these injured Australian apples was produced before or after the commencement of brown-heart. Evidently it is not, as yet, proven that brown-heart is a form of internal aldehyde poisoning. Further work is required, and has been planned, to attempt to determine whether carbon dioxide in conjunction with oxygen injures apple tissue either directly or indirectly other than by the induction of  $\text{CO}_2$ -zymasis.

#### SUMMARY AND CONCLUSIONS.

(1) In an earlier paper it was shown that ethyl alcohol and acetaldehyde, which are only present in traces in healthy Newton Wonder apples stored in air, are both formed during the respiratory processes which occur under anaerobic storage (*i.e.* by anaerobic zymasis), or

under the influence of certain mixtures of carbon dioxide and oxygen (*i.e.* by  $\text{CO}_2$ -zymasis). In that paper it was pointed out that both anaerobic zymasis and  $\text{CO}_2$ -zymasis were lethal processes, and that  $\text{CO}_2$ -zymasis was more lethal than anaerobic zymasis since in the former acetaldehyde, which is more toxic than ethyl alcohol, accumulates in greater concentration than in the latter<sup>(9)</sup>.

(2) In this paper the poisoning of apples of this variety by the products of zymasis has been considered in greater detail.

(a) Under anaerobic conditions these apples sooner or later will suffer from what has been called "invasive alcohol poisoning," associated with, although not necessarily caused by, an accumulation by anaerobic zymasis of more than 0.3 per cent. ethyl alcohol in the tissues. Never more than 0.006 per cent. acetaldehyde has been found in apples suffering from this injury, and the ethyl alcohol/acetaldehyde ratio is usually  $> 50/1$ . The incidence of this injury at the surface and its regular progress inwards are ascribed to the different susceptibility of the cells of apples to the adverse consequences of anaerobic zymasis.

(b) In gas mixtures containing carbon dioxide and oxygen, these apples either at once (if the concentration of carbon dioxide  $> 50$  per cent.), or in variable fashion according to their senescent states (if the concentration of carbon dioxide is  $< 30$  per cent. and  $> 20$  per cent.), undergo  $\text{CO}_2$ -zymasis, which is followed usually by "internal aldehyde poisoning" (when the concentration of acetaldehyde formed is  $> 0.01$  per cent.), and by "invasive aldehyde poisoning" (when the concentration of acetaldehyde formed is  $> 0.03$  per cent.). Never more than 0.08 per cent. of ethyl alcohol has been found in apples suffering from either form of aldehyde poisoning, and the ethyl alcohol/acetaldehyde ratio is of the order  $2/1$ . Evidence is given that these injuries are actually caused by acetaldehyde, and that their incidence in different parts of apples is due to the different susceptibility of the cells of apples to this substance.

(3) In gas mixtures containing carbon dioxide and oxygen, if the concentration of carbon dioxide is  $< 13$  per cent. (circa) and the concentration of oxygen is sub-normal, analyses of several varieties of apples indicate that zymasis does not occur; indeed, in such gas mixtures the storage lives of apples seem to be prolonged.

(4) With gas mixtures, containing carbon dioxide and oxygen, in which the concentration of carbon dioxide is  $> 13$  per cent. and  $< 20$  per cent., a type of internal browning called brown-heart sometimes occurs. Since it is still doubtful whether brown-heart is or is not a form of internal

## 456 *Ethyl Alcohol and Acetaldehyde produced by Apples*

aldehyde poisoning, experiments have been planned to determine more critically the relationship between brown-heart and zymasis.

(5) It may be possible in the future to diagnose invasive alcohol poisoning, and internal and invasive aldehyde poisoning, by determining the ethyl alcohol and acetaldehyde content of apples suspected by their appearance to be suffering from one or more of these injuries. Such analyses may be carried out many days after the occurrence of these injuries, since neither ethyl alcohol nor acetaldehyde is oxidised by apple cells, nor do they escape rapidly from apples. If these injuries were found in apples from stores in this country, or after transportation from overseas, it would follow from what has been said that the stores or ships' holds had not been properly ventilated. Indeed even if the apples are healthy any evidence of zymasis would point to the same conclusion. So analyses of this kind may prove useful in the future, if cases arise in which apples are suspected to have been damaged through neglect of rules as to ventilation.

### ACKNOWLEDGMENTS.

I wish to record my gratitude to Dr Franklin Kidd and Dr Cyril West of the Low Temperature Research Station, Cambridge, for their valuable criticisms during the progress of the work, and during the preparation of this paper. Some of the quantitative results which have been presented were obtained in the biochemical laboratory, Cambridge, and I am grateful to Prof. Sir F. G. Hopkins for the facilities given. To Mr Percy Gibson of the Botanical Department, Armstrong College, I am indebted for his willing assistance in taking photographs, and in many other ways.

### REFERENCES.

- (1) BROOKS and COOLEY (1917). *Journ. Agric. Res.* **xi**, No. 7, 287.
- (2) BROOKS, COOLEY and FISHER (Sept. 1920). *Farmers' Bulletin, U.S.A. Dept. of Agriculture*, No. 1160.
- (3) KIDD and WEST (1923). *Department of Scientific and Industrial Research, Food Investigation Board, Special Report No. 12*.
- (4) ——— (1925). *Ibid. Special Report No. 23*.
- (5) ——— (1927). *Ibid. Special Report No. 30*.
- (6) MAGNESS and DIEHL (1924). *Journ. Agric. Res.* **xxvii**, No. 1.
- (7) ONSLOW (1920). *Biochem. Journ.* **xiv**.  
—— (1921). *Ibid.* **xv**.
- (8) THOMAS (1925). *Ibid.* **xix**.





Fig 1 (*a*)



Fig 1 (*b*)



Fig 2 (*a*).



Fig 2 (*b*)



Fig 2 (*c*)



Fig 3 (*a*).



Fig 3 (*b*).



Fig 3 (*c*).



## EXPLANATION OF PLATE XX.

Figs. 1 *a* and 1 *b*. APPLES AFTER REMOVAL FROM ANAEROBIC CONDITIONS TO AIR, SHOWING EFFECTS OF INVASIVE ALCOHOL POISONING (see Section 1 (i)).

Fig. 1 *a*. Injury is incident in epidermal cells and progresses inwards.

Fig. 1 *b*. Finally the apple may be completely killed.

Figs. 2 *a*, 2 *b* and 2 *c*. BROWNING EFFECTS OF ALDEHYDE POISONING FOLLOWING CO<sub>2</sub>-ZYMESIS, EITHER IN WHOLE APPLES OR IN APPLES HALVED BY TRANSVERSE CUTS (see Section 2 (i)).

Fig. 2 *a*. Internal aldehyde poisoning, incident in the heart tissue, sometimes brought about by high concentrations of carbon dioxide in the presence of oxygen.

Fig. 2 *b*. Invasive aldehyde poisoning which is always produced in high concentrations of carbon dioxide in the presence of oxygen, and which appears after internal aldehyde poisoning, when this occurs. Here internal aldehyde poisoning is very severe.

Fig. 2 *c*. The apple may be completely killed by internal and invasive aldehyde poisoning.

Figs. 3 *a*, 3 *b* and 3 *c*. BROWNING EFFECTS PRODUCED BY THE VAPOUR OF ACETALDEHYDE ON APPLES HALVED BY TRANSVERSE CUTS (see Section 2 (i)).

Fig. 3 *a*. After short exposure to the vapour, showing the internal incidence of browning in the "heart" tissue (cf. Fig. 2 *a*).

Fig. 3 *b*. After longer exposure showing more intense "heart" browning, and commencement of invasive browning (cf. Fig. 2 *b*).

Fig. 3 *c*. The apple is eventually completely killed.

*(Received December 1st, 1928.)*

# THE INTERNAL CONDITION OF THE HOST PLANT IN RELATION TO INSECT ATTACK, WITH SPECIAL REFERENCE TO THE INFLUENCE OF PYRIDINE

By J. DAVIDSON, D.Sc.

*Waite Agricultural Research Institute, University of Adelaide, Australia,*

AND H. HENSON, B.Sc.

*Assistant Lecturer in Zoology, University of Leeds.*

*(From the Entomology Department, Rothamsted Experimental  
Station, Harpenden.)*

## PART I. THE NATURE OF THE PROBLEMS INVOLVED<sup>1</sup>.

By J. DAVIDSON.

THE study of the internal condition of the food plant in relation to insect attacks has received very little attention on the experimental side, and a wide field is open for research on these lines. After a lengthy investigation of the factors affecting the infestation of the tea plant by *Helopeltis theivora*, Andrews<sup>(1)</sup> concluded that the vitality of this insect is directly controlled by the suitability or otherwise of the food supply. Withycombe<sup>(11)</sup> in Trinidad has shown that sugar cane growing on heavy clay soils, having badly aerated roots and a marked susceptibility to water shortage, more readily succumbs to the attack of the frog-hopper *Tomaspis saccharina*. Davidson<sup>(3)</sup> showed experimentally that there was a marked variation in the intensity of reproduction of *Aphis rumicis* on *Vicia faba* depending upon the nutrients supplied to the plants. Lees<sup>(5)</sup> refers to several instances which indicate the varying susceptibility of plants to insect attack due to the influence of soil conditions and growth of the plant.

The association of particular species of insects with particular food plants has doubtless resulted in an adaptation on the part of the insect, especially with regard to the physiology of digestion, in a manner best suited to its requirements. Many insects fail to live, or at any rate develop abnormally and only multiply at a markedly reduced rate, on

<sup>1</sup> The observations and experimental work on this subject were made at the Rothamsted Experimental Station while the writer was a member of the staff of the Entomological department.

other than their normal food plants as the writer(2) has shown to be the case with the polyphagous aphid *A. rumicis*. In some cases the specificity of the food plant is so definite that certain insects inhabiting one variety of a plant will fail to live on a closely related variety. One of the best known instances of this is the resistance of certain varieties of vines to Phylloxera. Trägårdh, as referred to by Müller(6), records that apple trees may differ from 2.5 to 80 per cent. in the degree to which they are attacked by *Argyresthia conjugella*.

The resistance or immunity of a plant to insect attack may be due to particular features of its morphology or anatomy. In most instances, however, it appears to be due to features closely associated with the physiology of the plant, probably the presence or absence of particular substances in the tissues of the plant which render it unsuited to the insect. For instance, the larvae of *Pieris brassicae* and *P. rapae* appear to feed on those plants whose leaves contain a particular glucoside, one of the mustard oils, and it has been shown by Verschaffelt that when this substance was smeared on leaves which the caterpillars normally avoid, they were attracted to feed on them. The chemotropic influence and other factors which enable adult insects to select their appropriate food plants is little understood, and would be a profitable line of enquiry.

It is interesting to note that certain alkaloids, which when extracted from plants are toxic to insects, are not so in their natural state in the living plant. For instance, the tobacco plant is visited by cut worms, caterpillars and plant-sucking insects, yet nicotine which is extracted from it is a potent insecticide.

It may well be that the presence of certain chemical substances in the juices of plants render them unsuited to the requirements of certain insects, but no clear experimental proof of this with reference to insect-attack, so far as I know, has been brought forward. By inoculating the juices from one plant containing particular chemical compounds into another plant known to be lacking in those substances, one might expect the substances would be taken up into the juices of the latter plant, at any rate in so far as its physiological reaction would allow of this taking place. With this point of view, Müller(6) injected extract of tomato rhubarb and tobacco sap into the stem of an apple tree infested with *Eriosoma lanigerum* in May and June, but there was no evidence that the aphid was affected by this treatment.

Grafting would appear to be a more reliable method of obtaining a transference of particular substances from one plant to another. Roach(8), who cites several authors, states, "It is known that grafting together

portions of two different plants is sometimes followed by changes in their chemical contents owing to permeation of each by substances formed in the other." He refers to the work of Brown (1926), who showed that the mineral composition of the fruit of the apple varies according to the stock on which the variety is grafted, and Barker and Grove (1914), who found that the acidity and probably the tannin content are similarly affected.

The so-called "*incompatibility*" between scion and stock, when for instance certain varieties of pears are grafted on certain quince stocks, which is being investigated at East Malling Research Station is of interest in this respect. The results of research on the physiological and biochemical aspect of grafting should be of great interest to entomologists in relation to the association of certain insects with particular varieties of fruit trees.

In a recent paper Dontaho Kostoff<sup>(4)</sup> states: "In grafting certain species and genera of the Solanaceae, mutual induction of antibodies in scion and in stock was found." "Physiological interaction between scion and stocks accompanied by different morphological phenomena in the scions and stocks were observed, which are explicable by postulating antibody production."

From the entomological standpoint, attempts to confer on plants temporary immunity from insect attack have been based on the view that the plant may be induced to take up, and absorb into its tissues, traces of certain chemical substances which, although innocuous to the plant concerned, would by their presence render it unsuited or even toxic to the insects infesting it.

It is clear that the results of any attempt to influence the growing plant as indicated above must be closely bound up with the physiological processes of the plant, and it is not surprising to find that conflicting results have been obtained by different investigators. In a recent monograph entitled *Die innere Therapie der Pflanzen*, A. Müller<sup>(6)</sup> has reviewed the subject very thoroughly and shown how complicated are the many factors concerned. This monograph is a noteworthy contribution to a wide subject which has been comparatively little explored.

The methods which have been employed by various workers in introducing chemical substances into plants may be briefly grouped under two headings:

(a) By injection of substances in solution into the stem or roots of plants (usually trees) or by introducing solid substances (*e.g.* potassium cyanide) into holes in the stem.

(b) By introducing the substances either as solids or as solutions into the medium in which the plant is growing, the substances being taken up in the usual way through the roots of the plant.

The former method has been used by several workers with the object of ridding trees of borers, aphids, and scale insects. In the case of solutions, holes are bored into the stem in which a tube is inserted, the solutions being led into the tree from a suitable reservoir. The staining of the wood of living trees on this principle has long been practised and various types of apparatus have been devised for introducing solutions into trees. Under the influence of the transpiration current, a fairly uniform distribution of the solutions throughout the tissues is obtained. The wood when seasoned is used for making furniture. Aniline dyes and those of the phenol group have been chiefly used at concentrations of 0.5 to 1 per cent., about 600 litres of solution being required per cubic metre of the tree. Dementiev (*Abstr. R.A. Entom. A.* III, 1915, 394) introduced a solution of barium chloride 1 : 350 into apple trees infested with woolly aphid in July 1903. He records that after nine days the aphids had been practically all killed. Müller<sup>(6)</sup> tried similar methods against *E. lanigerum* using solutions of chloral hydrate, nicotine muriate, and pyridine. The latter gave good results. Marked branches of apple trees treated with pyridine on 8. vi. 22 were found to be freed from the aphids on 7. vii. 22.

Employing similar methods, other chemical substances in solution have been tried by different workers, but in many cases the results have either been negative, or the resulting damage to the plants, due to the treatment, has ruled out the method as of no practical value. It is clear, however, that meteorological conditions, time of the year (season), age and type of tree, and concentrations of the substances used, are important factors affecting the results obtained.

The introduction of solid substances into the stem of the plant has the advantage of being a convenient and simple method. Sanford (*Science*, Oct. 9th, 1914, No. 1032, 519) treated Spanish broom infested with *Icerya purchasi* by boring a hole three inches deep, diameter three-eighths inch, into the stem and filling it with crystals of potassium cyanide. In a few days the scale was killed and the tree flourished. Shattuck (*Science*, Feb. 26th, 1915, No. 1052, 324) states that large groves of elms and black locust trees were successfully treated by this method against attacks of borer and girdling insects. Moore and Ruggles (*Science*, July 2nd, 1915, No. 1070, 33) however obtained negative results with this method against wood borers in oak trees. Müller (p. 49)

refers to the work of Mokrzecki, who successfully treated apple and pear trees infested with *Lepidosaphes ulmi* and *Diaspis fallax* with iron sulphate solution.

The method whereby the chemical substances concerned are added to the soil in which the plants are grown is a more normal and convenient one. There are some interesting examples in the literature showing the curative effect on plants of adding particular substances to the soil. The best known case is probably that of chlorosis due to deficiency in the soil of iron or manganese. Samuel and Piper<sup>(9)</sup> have shown conclusively that the "Grey Speck" disease of oats in certain areas of South Australia is due to a manganese deficiency in the soil, and by the incorporation of manganese sulphate in the soil the disease can be completely cured. Andrews<sup>(1)</sup>, working with *Helopeltis theivora* infesting the tea plant in North-east India, found "That when a constant supply of soluble potash is applied to the roots of the (tea) bush, this substance is taken up and the bushes which are entirely shut up by the pest can be caused to throw it off entirely, and remain immune from attacks for the rest of the season." Brenchley and Warrington at Rothamsted and others have shown the stimulating effect on plant growth of minute doses of certain chemical substances, e.g. Boron, which in slightly stronger doses are toxic to the plant.

Müller<sup>(6)</sup>, working with "Blumenbohnen" in water culture, tested the effect on the plants of a range of substances in various concentrations. He found for instance that with pyridine 1 : 500 and barium chloride 1 : 5000, after 5 and 17 days respectively, no ill-effects were noted on the plants<sup>1</sup>.

In 1924 the writer made some preliminary experiments in which solutions of various substances were inoculated by means of a hypodermic syringe into the stems of growing plants of *Vicia faba* infested with aphids. The following substances were used, strychnine, atropine, digitalin, arsenic, potassium cyanide, nicotine, and pyridine. In several cases the substances were evidently carried through the plant in an irregular manner to the growing tip, and the aphids were killed. In some cases the plant itself was also killed, pathological symptoms first showing in certain areas of the growing tip, and as in the case of arsenic gradually spreading through the whole plant. Experiments were continued the

<sup>1</sup> With  $\text{MgSO}_4$  1 : 500 and chloral hydrate 1 : 1000, it was observed that the plants made better growth and bigger root development. The writer<sup>(3)</sup> working with *Vicia faba* in water culture in 1923 also obtained marked increase in root development and growth of the plants in solutions containing increased  $\text{MgSO}_4$  as compared with the controls growing in normal culture solution.



following year by placing cut stems of *Vicia faba* plants infested with *Aphis rumicis* into solutions of various substances. The examples given in the following table of some results obtained at Rothamsted in July 1926 by Mr H. T. Pagden indicate the type of results obtained.

Solutions used	Plant no.	Date and time observations made					
		28. vii. 10 a.m.	29. vii.		30. vii.		
			10 a.m.	6 p.m.	10 a.m.	6 p.m.	
Tap water controls	1	Bean plants placed in solutions 10 a.m. Plants 12-14 in. in height	Plants and aphids normal	Normal	Normal, aphids quiet	Normal, aphids quiet	
	2						
	3						
KCN 0.25 %	4		Aphids slightly wandering over plants; plants appear normal	Aphids sluggish; plants wilting and bending over about middle of stem	Aphids sluggish; plants wilting	Many aphids dead; did not wander, but died with stylets in tissues of plant	
	5						
	6						
BaCl <sub>2</sub> 1 : 350	7		Aphids wandering, some dead; plants wilting	—	Some aphids dead, many wandering actively; plants with leaves badly blotched	—	
	8						
	9						
MgSO <sub>4</sub> M/10	10		Plants and aphids normal	Plants normal; aphids slightly wandering	Plants appear normal; aphids wandering	Plants appear normal; aphids actively wandering over plants and muslin cover	
	11						
	12						

*Note.* 100 c.c. of solution used in each case. Mean temperature throughout the period was 24.5° C. The amount of solution taken up by the plants varied. In the case of the control series, 50 c.c. were taken up during the first 24 hours, whereas in the case of the other solutions only 15-20 c.c. were taken up.

From preliminary experiments with pyridine, it was found that, when beans grown in sand culture and infested with *Aphis rumicis* were watered with pyridine solution 1 : 200 and 1 : 150, the pyridine was evidently taken up by the plant to the growing tip, and the aphids wandered from the plant about 12 hours later, the great majority of them being killed. During the first day or two after commencement of the experiment, the plants did not exhibit any marked effect of the pyridine. On examination of the roots after a few days it was found, however, that the fine rootlets were discoloured and dead. When the pyridine solution was watered to beans growing in soil, the effect on the aphids was not so quickly shown. In order to find out the strength of pyridine solution required to free the plants from the aphids and at the same time not to injure the plants, Mr Henson has carried out a series of experiments which are described in the second section of this paper. The results obtained indicate a useful line of enquiry in relation to the effect of pyridine and other chemical substances on the aphids and the physiological reaction of the plant.

## REFERENCES.

- (1) ANDREWS, E. A. (1923). *Factors affecting the control of the Tea Mosquito Bug*. Indian Tea Assn. London.
- (2) DAVIDSON, J. (1921). *Ann. App. Biol.* vii, 51-65.
- (3) — (1925). *Ibid.* xii, 472-507.
- (4) DONTAHO KOSTOFF (1929). *Genetics*, xiv, No. 1, 37-77.
- (5) LEES, A. H. (1926). *Ann. App. Biol.* xii, 506-515.
- (6) MÜLLER, A. (1926). *Die innere Therapie der Pflanzen*. Monogr. zur angew. Entom. No. 8, Paul Parey, Berlin.
- (7) — (1926). *Anzeiger für Schädlingskunde*. Jahrg. ii, Heft 12, 157-164.
- (8) ROACH, W. A. (1927). *Ann. App. Biol.* xiv, 181-191.
- (9) SAMUEL, G. and PIPER, C. S. (1928). *Journ. Agric. South Australia*, xxxi, 696, 789.
- (10) WILKINS, V. E. (1927). *Research and the Land*. H.M. Stationery Office, London.
- (11) WITHEYCOMBE, C. L. (1926). *Ann. App. Biol.* xiii, 64-108.

PART II. ON SOIL TREATMENT WITH PYRIDINE AND  
ITS EFFECT ON THE INFESTATION OF *VICIA FABA*  
BY *APHIS RUMICIS* L.

By H. HENSON.

Dr Davidson's preliminary experiments on this subject are referred to in Part I of this paper. They indicated that it was possible to administer a substance such as pyridine to a plant and that it would take this up *via* the roots and so render itself toxic to insect pests such as aphids, which live by sucking the plant juices. During the summer of 1928 a series of experiments were undertaken in order to further investigate this subject and to determine whether the method showed promise of being of any economic importance. Pyridine was chosen because it was known to give positive results and because its odour rendered its presence in the plants easily detectable. A wide field for investigation is offered by the possibility of using other substances. The whole work was done under the direction of Dr A. D. Imms and Dr J. Davidson, to whom my best thanks are due.

Three experiments were projected as follows:

(a) Administration of pyridine to infected broad beans under conditions which would prove whether or not a lethal effect, due to root absorption, could be obtained. It was also used as a preliminary investigation of the degree of concentration required.

(b) Administration of pyridine under conditions similar to the above, to determine whether a range of concentrations was available in which the pyridine was potent to the animal but harmless to the plant.

(c) An experiment in which beans not infected with aphids were given pyridine and then its effect determined by taking the dry weights of the plants treated, and of controls untreated. This experiment was also taken advantage of to determine whether a small amount of pyridine administered several times had a different effect from a larger amount administered once.

### *Experiment 1 (a).*

Two series of beans were grown, one in soil and the other in sand. The sand cultures consisted of 48 pots divided into five series, four of ten each and one of eight. The pots were  $9 \times 11$  in. (inside measurements) and were each fitted with a cork and glass drain tube. Any water which drained through was always returned to its own particular pot. Three days after planting (May 11th) each pot was given 1 gm. potassium nitrate, 1.24 gm. sodium sulphate, 0.4 gm. calcium chloride, 0.5 gm. magnesium sulphate, 0.5 gm. potassium dihydrogen phosphate and a trace of iron chloride made up in tap water. Subsequently the plants were only watered as required. On May 30th each plant was infected with one apterous viviparous female aphid (*A. rumicis* L.), the plants covered with muslin bags, and the infestation allowed to proceed. The commencement of the experiment for each pot was taken as the day on which the first young were produced. Apart from a few of the controls each pot was given 18 days as a reproduction period taking the day on which the first young were produced as the starting point. On June 11th pyridine was administered to 30 of the pots. The five series into which the 48 pots were divided were as follows:

1. Ten control pots in which petri dishes containing pyridine (1 part pyridine in 200 parts water) were put on the surface of the sand as a check on the action of the vapour given off from the treated pots.
2. Eight control pots in which dishes were not present.
3. Ten pots to each of which 500 c.c. of a  $\frac{1}{2}$  per cent. solution of pyridine in water were added, i.e.  $2\frac{1}{2}$  c.c. pyridine per pot.
4. Ten pots to each of which 500 c.c. of a  $\frac{1}{4}$  per cent. solution of pyridine in water were added, i.e.  $1\frac{1}{4}$  c.c. per pot.
5. Ten pots to each of which 500 c.c. of a  $\frac{1}{8}$  per cent. solution of pyridine were added, i.e.  $\frac{5}{8}$  c.c. per pot.

## 466 *Condition of Host Plant in Relation to Insect Attack*

The controls were given 500 c.c. of water at the same time as the pyridine was administered to the other pots.

A table of the results is given below. The reproduction figures are given in the columns. Owing to pressure of time and the fact that pots were urgently required for the next experiments, some of the controls were examined before the expiry of their allotted 18 days. These are indicated in the columns.

Exp. 1 took place over the period May 31st-June 23rd. Mean temperature during this period was 60° F.

Table I.

	Controls		Pyridine administered		
	With vapour dishes	Without vapour dishes	½ c.c. per pot	1½ c.c. per pot	2½ c.c. per pot
1	388 (15 days)	365	0 d	0 d	0 w
2	391 (17 days)	385	9	0	0 w
3	398	396 (13 days)	15	0	0 d
4	413 (13 days)	411 (13 days)	46	0	0 d
5	421 (16 days)	—	59	2	0 w
6	510	566	66	6	0 w
7	518	—	70	12	0 d
8	820	747	75	23	0 d
9	878	782	169	32	0
10	Discarded	1043	295	58	4
Mean	526	587	80	13	—

It will be seen at once that the pyridine has a very marked effect on the infestation. In those cases in which the infestation was not entirely checked it seemed likely that it was due to reinfection after wandering of the aphids. Very numerous dead aphids were to be observed on all the plants which were given pyridine. The figures further indicate that the plant takes up the pyridine in direct proportion to the concentration in which it is administered.

*Notes on the effect on the plants.* Too much significance should not be placed on the following notes as the pyridine was in contact with the plant roots for a matter of five days or so, which would not be the case under field conditions. A small "w" in the columns in Table I indicates the plant to have been badly wilted. A small "d" indicates less severe damage. The roots were badly affected in all cases, being caused to go black and lose their turgidity. The effect was worse in the higher concentrations as would be expected. The wilting was probably correlated with cessation of absorption by the roots. The affected plants also tended to go lighter in colour. Of course the controls were not affected

in this manner. In a few plants the pyridine was washed out and the plants allowed to go on developing. One of these bore fruit and was still healthy on July 24th.

*Experiment 1 (b).*

Soil pots were treated in a similar fashion. Twenty-four pots were taken and divided into three series: (1) 8 controls; (2) 8 to which  $\frac{5}{8}$  c.c. pyridine per pot was administered in 500 c.c. water; (3) 8 to which  $2\frac{1}{2}$  c.c. pyridine per pot were administered in 500 c.c. of water.

Beans planted May 9th. Plants infected May 30th. Allowed 18 days as time of reproduction.

Results tabulated below (Table II).

Table II.

*Soil series.*

Controls	Pyridine administered	
	$\frac{5}{8}$ c.c. per pot	$2\frac{1}{2}$ c.c. per pot
1214	1202	50
1211	915	24
906	866	12 d
882	733	11
390	618	10
214	209	3
1281	128	2
Discarded	Discarded	0
Mean	871	14

Mean temperature 60° F.

It will be seen that only one plant was badly affected. In fact all the others were apparently normal. The roots also were not nearly so badly affected as in the sand pots.

Exp. 1 proves that the pyridine is absorbed by the plants and, provided a suitable concentration is used, is lethal to the aphids. The concentrations to be used in Exp. 2 lie between  $2\frac{1}{2}$  c.c. and  $\frac{5}{8}$  c.c. per pot in the soil series, and between  $1\frac{1}{2}$  c.c. and a lower limit not determined by Exp. 1 in the sand pots.

*Experiment 2 (a).*

In Exp. 2 (a), thirty sand pots were taken as before. The beans were planted in seed boxes on May 31st, transplanted to the pots on June 19th and culture media given to each pot as in Exp. 1, *i.e.* 1 gm. potassium nitrate, 1.24 gm. sodium sulphate, 0.4 gm. calcium chloride, 0.5 gm.

## 468 *Condition of Host Plant in Relation to Insect Attack*

magnesium sulphate, 0.5 gm. potassium dihydrogen phosphate made up in tap water. On June 25th each plant was infected with a young apterous form and left to become infested. The date on which each mother first produced young was noted and the experiment was continued for 16 days (each pot) after this date. Pyridine was administered to each pot (in 500 c.c. of water) on the twelfth day (after infection) and left in contact for four days. The pots were then washed through with tap water.

Six series of five plants each were taken:

- (1) Controls to which no pyridine was given.
- (2) Five pots to which 0.1 c.c. pyridine was given.
- (3) Five pots to which 0.2 c.c. pyridine was given.
- (4) Five pots to which 0.5 c.c. pyridine was given.
- (5) Five pots to which 0.6 c.c. pyridine was given.
- (6) Five pots to which 1.25 c.c. pyridine were given.

During the period of the experiment the temperature was abnormally high and a high reproduction rate prevailed among the aphids. Under these conditions any slight variation in the action of the pyridine produced a much more noticeable effect on the counts due to the very rapid recovery after the check on the infestation. This is suggested as the explanation of the rather aberrant figures of 617 and 580 in two of the 1.25 c.c. pots. Further, it is probable that under the hot dry conditions the plants absorbed and quickly eliminated the pyridine. No data have been obtained with regard to variation in rate of absorption by the plants or of the conditions of their responses to the presence of the poison.

A table with the infestation figures is given as before.

Reproduction period 16 days. Mean temperature July 2nd-21st 70° F.					
Controls	·1 c.c. per pot	·2 c.c. per pot	·5 c.c. per pot	·6 c.c. per pot	1.25 c.c. per pot
2869	1584	681	667	181	617
2655	1842	1311	290	50	580
2297	1691	1463	415	223	56
2562	1657	Plant no good	554	242	Plant died
2908	2757	1739	206	56	3
Mean:					
2658	1906	1298	426	150	314

The actual concentration per pot cannot be ascertained owing to the amount of water in each pot being unknown. The lowest concentration used was 0.1 c.c. per pot and was actually something less than 1 in 5000.

It will be observed that the same effect was obtained as in Exp. 1. The effect on the aphids is proportional to the concentration of the pyridine. This is the case even down to the very lowest concentrations used. No clear cut range of concentrations has been obtained in which the pyridine was lethal to the aphids but harmless to the plants.

*The effects on the plants.*

In no case was a detrimental effect on the plants noticed. As the highest concentration used was 1.25 c.c. per pot this agrees almost exactly with what was found in Exp. 1, where only one plant was damaged seriously by this concentration. From casual observance there seems to be a check on growth but no actual damage. Ten plants were washed clear of pyridine and left. These were still alive and healthy on August 18th.

From these data it would seem that two courses of action could be taken to check infestation: (a) a low concentration administered several times, or (b) a high concentration (represented by the 1.25 c.c. per pot conc.) allowed to act once.

Whichever of these alternatives is chosen will depend on the effect on the plants. Further data on this point will be obtained from the dry weight experiment.

*Experiment 2 (b).*

This was another soil pot experiment which gives results in accordance with what was discovered in Exp. 1 (b).

Reproduction period 16 days. Mean temperature 70° F.

	Controls	.8 c.c. per pot	1.25 c.c. per pot
	1496	538	544
	1123	227	1237
	333	2107	649
	1414	528	72
Mean	1091.5	850	625

*Experiment 3.*

The object of this experiment was to determine the effect of the pyridine on the plants. Beans were planted in sand pots on June 20th. On June 21st each pot was given a culture solution containing 3 gm. potassium nitrate, 3.72 gm. sodium sulphate, 1.2 gm. calcium chloride, 1.5 gm. magnesium sulphate, 1.5 gm. potassium dihydrogen phosphate (made up in tap water). Each pot had ample supplies of water.

## 470 *Condition of Host Plant in Relation to Insect Attack*

Twenty-four pots were used and divided into three series as follows:

- (1) Eight controls to which pyridine was not added.
- (2) Eight pots to which 1.2 c.c. pyridine in 500 c.c. water were added on the fortieth day after planting (July 30th).
- (3) Eight pots to which 0.2 c.c. pyridine in 200 c.c. water was added on six occasions, July 24th, 26th, 28th, 30th, August 1st and 3rd.

Each pot had a drain tube as in previous experiments and ample supplies of water were always present; the drain water was returned to the pot at intervals.

The results are given in the table as dry weights of the plants concerned.

Dry weights. Period of experiment June 20th–August 14th. Weight in gm.

	Controls	1.2 c.c. per pot given once	.2 c.c. per pot given six times
	1.9	1.45	1.55
	3.95	2.15	1.7
	4.2	2.85	2
	4.45	3	2.85
	5.3	3.5	3.9
	6.7	3.6	4.5
	8.3	4.7	4.95
	9.3	5.55	5.05
Total	44.1	26.8	26.5
Mean	5.5	3.35	3.31

It will be seen at once that a very marked effect on the plants is evident from their dry weights. This single experiment is not by any means conclusive, since the existence and extent of the subsequent recovery remain unknown and many more experiments are really required to settle these points. In this particular case no difference is to be observed between the series in which a large dose of pyridine was administered once and the series in which an equivalent amount was administered in successive small doses.

### CONCLUSION.

It is known that it is possible to administer to the roots of broad beans certain substances which are absorbed and transferred to the leaves and stems. In experiments in which pyridine was used it is shown that this substance, in suitable concentrations, exercises a marked detrimental effect upon the aphids. There are many features which render the exact conditions rather difficult to define. The great majority of these features are concerned with the conditions governing absorption



by the plant and with the effect of the pyridine on the plant after absorption. It was noticeable in the sand experiments that the effect on the aphids was to a large extent proportional to the amount of pyridine administered to the plant. The experiment in which dry weights of treated and control plants were compared shows quite plainly that a very evident effect is caused by the pyridine. In experiments on plants growing in soil the pyridine appeared to have a much less detrimental effect on the plants. It still had a very obvious effect on the aphids when present in sufficiently high concentration. This concentration had to be much higher than was the case with plants grown in sand.

*(Received April 19th, 1929.)*

## CARBON DIOXIDE PRODUCTION IN SANDS AND SOILS IN THE PRESENCE AND ABSENCE OF AMOEBAE

By D. WARD CUTLER, M.A. AND L. M. CRUMP, M.Sc.

*(From the General Microbiology Department, Rothamsted  
Experimental Station, Harpenden.)*

(With 2 Text-figures.)

If the functions of the micro-organisms in soil are to be fully understood a knowledge of their relation to the different chemical constituents of the soil is essential, so that the parts they play in breaking down or building up compounds can be evaluated. The present work was undertaken in an attempt to throw some light on the relation of bacteria and amoebae to carbon dioxide production and by this means to the organic matter in the soil. Such investigation is particularly interesting since it has been shown that amoebae are an important part of the active soil population and that their numbers are definitely related to those of the bacteria (4). Since the problem is one of great complexity only the simplest cases have hitherto been considered and the attempt has been confined to studying the action of one pure strain of bacteria, in the presence or absence of a pure line of amoebae, in sand cultures containing four different carbon sources, and in sterilised soils with four different manurial histories.

Previous work on carbon dioxide production from soil has largely been directed to attempting to correlate for field soils the carbon dioxide produced with their fertility, no effort being made to simplify the population; the literature of this subject is reviewed by Waksman and Starkey (15) and Waksman (14) and need not be considered here. All the work done has gone to show that carbon dioxide formation is conditioned by numerous factors, among which heat, moisture, source of carbon and type of organisms may be mentioned as the more important, and many investigators have concluded that it is safe to regard carbon dioxide production as a useful index of soil fertility, possibly of greater use in this connection than the actual numbers of bacteria present in the soil. Several observers (Stocklase (12), Russell and Appleyard (10), Neller (7),

König and Hasenbäumer<sup>(8)</sup>, van Suchtelen<sup>(13)</sup> found a definite correlation between carbon dioxide production and bacterial numbers. The amount of carbon dioxide produced by an unsterilised field soil is very variable; we have found that a well manured soil such as the dunged plot on Barnfield can give from 18 to 157 mgm. per kilo for the first 24 hours of an experiment, and an unmanured, 12 to 32. Waksman and Starkey recorded over 100 mgm. from one kilogramme of soil from a well manured, limed plot, and about 20 mgm. from an untreated soil.

A certain amount of work has also been done with pure cultures of bacteria inoculated into field soils; Westhues<sup>(16)</sup> found that sterile garden soil inoculated with three different species of bacteria gave amounts of carbon dioxide varying from 136 to 274 mgm. per 500 gm. in seven weeks.

Very little work has been done on the respiration of protozoa, one can therefore lay down no *a priori* expectation as to the amount of carbon dioxide that they will be responsible for producing in the soil. The little that is known is mostly derived from work on *Paramoecium*. Barratt<sup>(1)</sup> calculated that *Paramoecium* gave off from 1.3 to 5.3 per cent. of its body weight per day, and Pütter<sup>(9)</sup> calculated that this would represent 0.000035 mgm. per animal per day, but it must be remembered that Barratt's animals were in distilled water so that metabolism was at a low ebb and the figures quoted are therefore minimum and not average.

#### METHODS.

In the experiments under consideration both sands and soils were used as medium; the sand was silver sand which passed a 1 mm. sieve, it was washed with hydrochloric acid, and then with water until it was acid-free, and was then ignited. To the sand four different food sources were added in different experiments, after sterilisation, when the micro-organisms were inoculated. The four food solutions used were soil extract, mineral salt solution + 0.5 per cent. peptone, mineral salt solution + ammonium sulphate + 0.2 per cent. glucose (ratio C/N = 10), and mineral salt solution + sodium nitrate + 0.2 per cent. glucose (ratio C/N = 10).

The soils were taken from plots on Barnfield which have been under continuous cultivation since 1876, receiving respectively fourteen tons of farmyard manure, 550 lb. nitrate of soda and 400 lb. ammonium salts per acre per annum; the fourth plot received no manure. The soils all received the same treatment in the laboratory; they were air-dried, sieved through a 3 mm. sieve and the required amount was then autoclaved

## 474 *Carbon Dioxide production in Sands and Soils*

in a two-litre flask for ten minutes at 15 lb. pressure. The sand was similarly autoclaved. Experience has shown that soil which is to be used as a medium for the growth of protozoa must not be subjected to a too drastic sterilisation, since toxic products are formed which inhibit growth<sup>(3)</sup>. Pure cultures of "YB" bacteria<sup>(3)</sup> were employed, obtained from a single cell<sup>1</sup>, either with or without the addition of a pure mixed culture of *Hartmanella hyalina* with "YB," and every experiment consisted of parallel cultures of bacteria alone and bacteria + amoebae, so that the results are strictly comparable. The media were inoculated under aseptic conditions by spraying the cultures of bacteria or bacteria and amoebae on to the sand or soil from an Atlas spray, sufficient food solution in the case of sands or mineral salt solution in the case of soils being added to bring the medium to half the water-holding capacity. Bacteria and amoebae were counted daily by the dilution method in use in this laboratory<sup>(4)</sup>.

The carbon dioxide evolved was estimated by the Pettenkoffer method using a baryta solution of about 0.1 per cent. in the tubes and titrating it against known hydrochloric acid of not more than  $N/5$  strength. In every case the cultures were aerated by drawing carbon dioxide free air over them continuously by an aspirator. It has been shown by Potter and Snyder<sup>(8)</sup> that within wide limits the rate at which the air is drawn over the soil is immaterial; in these experiments the rate was between four and five litres in 24 hours. The carbon dioxide was estimated at least once in every 24 hours, that is, when the bacteria and amoebae were counted, but frequently the amount produced was so great as to necessitate more frequent titrations.

### RESULTS.

The growth of bacteria in an inoculated sand or soil follows closely the growth in an ordinary liquid culture; the numbers rise to a maximum within the first five days and then fall steadily with minor fluctuations; the presence of amoebae induces a greater tendency to fluctuate and lowers the bacterial numbers. Contrary to expectation, in the majority of cases the carbon dioxide production reaches its maximum a day before the bacterial numbers have reached theirs; then it drops sharply at first and more and more slowly until a steady level is maintained, at least for the duration of the experiments under discussion, which is a period of anything up to 15 days. Typical curves showing the carbon

<sup>1</sup> This pure strain of bacteria was prepared for us by Mr R. H. Stoughton using the "Dickinson Micro-Isolator."

dioxide production are given in Figs. 1 and 2. The amount of carbon dioxide produced in any given period is conditioned by a variety of factors as has been previously stated, but a rough idea of the amount

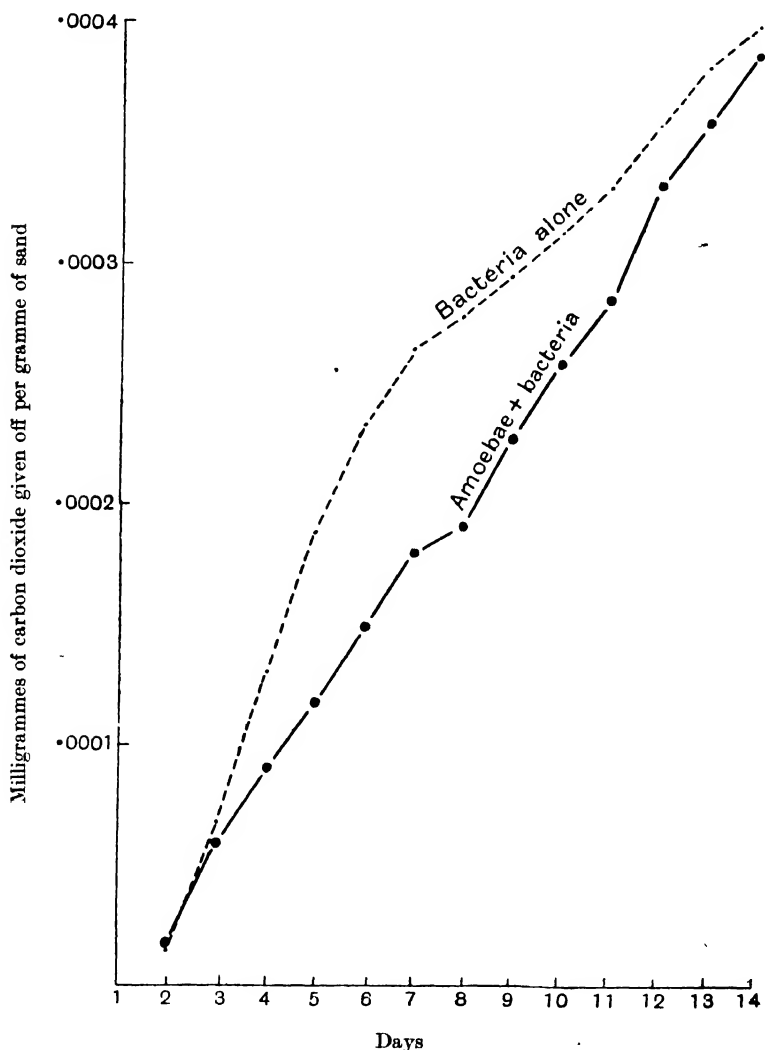


Fig. 1. Carbon dioxide production from bacteria and bacteria + amoebae in sand cultures with peptone.

of carbon dioxide which any given medium is capable of producing may be obtained from Table I, in which the amount of carbon dioxide given off during the first five days of an experiment is given.

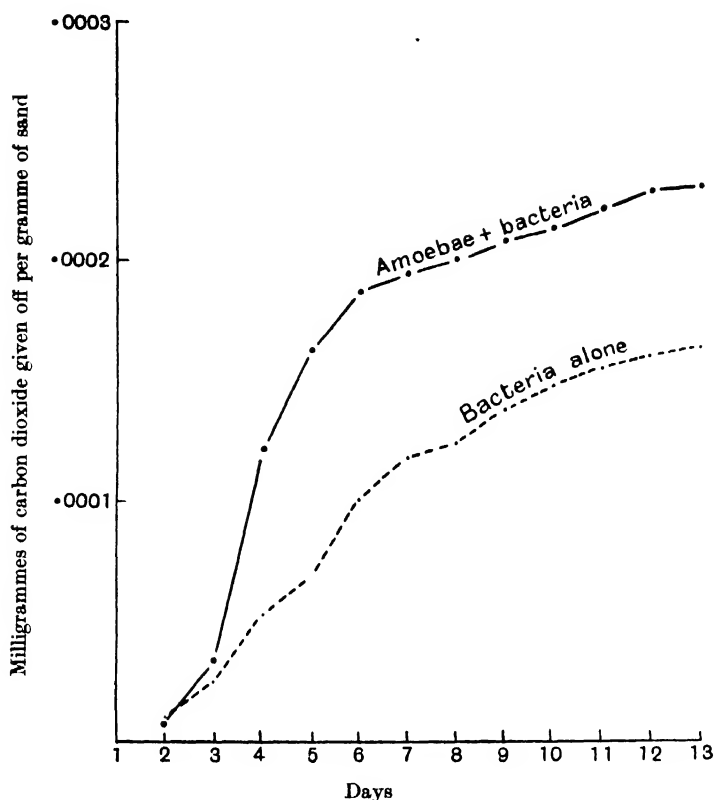


Fig. 2. Carbon dioxide production from bacteria and bacteria + amoebae in sand cultures with ammonium sulphate and glucose.

Table I.

*Average amount in gm. of carbon dioxide produced from 400 gm. of media in five days.*

Soils	Farmyard manured	Unmanured	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	NaNO <sub>3</sub>
	.1984	.0324	.0592	.0556
Sands	Peptone	Soil extract	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	NaNO <sub>3</sub>
	.1126	.0164	.0222	.0212

It is obviously of considerable interest to know what relation the numbers of bacteria bear to the formation of carbon dioxide; usually it has been assumed that there will be a definite relation between high numbers of bacteria and high carbon dioxide production and *vice versa*. The simplest method of testing this point since only two variables are involved is to arrange the data in contingency tables: in these an increase

in either variant is shown by a + sign and a decrease by a - sign; if both increase or decrease together it will be shown by a ++ or a --, if they vary inversely by a +- or -+. Where the two vary wholly independently there will be equality between the numbers of like and unlike signs, provided that there are a sufficient number of cases; a preponderance of like or unlike signs will show that the two variables are related to one another. To test the significance of any departure from equality a  $\chi^2$  may be worked out (5), and when a  $\chi^2$  has a greater value than 4 it may be assumed that there is a definite inter-relation between the two variables which is not due to chance. From Table II it appears

Table II.

*Contingency tables for carbon dioxide production and bacterial numbers in sand and soil cultures (CO<sub>2</sub> given first).*

Sands		++	+-	+ -	--	$\chi^2$
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> or NaNO <sub>3</sub> + glucose	Bacteria alone	11	5	3	12	6.2
	With amoebae	10	4	6	13	5.1
Peptone	Bacteria alone	12	6	6	15	5.6
	With amoebae	10	8	10	12	0.4
Soil extract	Bacteria alone	15	7	4	11	6.1
	With amoebae	12	15	6	9	0.08
Soils		++	+-	- +	--	$\chi^2$
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> or NaNO <sub>3</sub>	Bacteria alone	9	20	9	17	—
	With amoebae	11	12	8	13	—
Farmyard manured	Bacteria alone	12	6	4	23	12.9
	With amoebae	7	6	3	13	3.9
Unmanured	Bacteria alone	3	8	5	7	—
	With amoebae	1	3	2	3	—

that where bacteria alone are present in sand cultures there is a very definite correlation between their numbers and the amount of carbon dioxide given off. The same is true of the soil receiving farmyard manure, but in the case of the unmanured soil and the soils receiving minerals there is no such correlation. There is no obvious reason for this anomalous result.

It is a matter of considerable interest to discover whether the bacterial capacity for producing carbon dioxide varies at all in the course of growth, and on different media, or whether it remains a constant. It appears from a study of different experiments that the carbon dioxide is very often produced in large quantities at the beginning of an experiment, even where in some cases the numbers of bacteria are still low. A contingency table was therefore made in which the two variables considered were the numbers of bacteria per gramme of medium,

## 478 *Carbon Dioxide production in Sands and Soils*

averaged for the beginning and end of each 24-hour period, and the amount of carbon dioxide produced per bacterium during that 24 hours, this giving a measure of the efficiency of the bacteria.

Table III.

*Contingency table for numbers of bacteria and efficiency in producing carbon dioxide (efficiency given first).*

Sands	++	+-	-+	--	$\chi^2$
Soil extract	10	13	14	5	4.8
Minerals + glucose	4	10	8	7	1.9
Peptone	5	10	11	4	4.8
All media	19	33	33	16	9.5
Soils	++	+-	-+	--	$\chi^2$
Farmyard manured	6	9	14	8	2.0
Unmanured	3	5	12	3	4.1
Minerals	6	12	27	11	7.1
All media	15	26	53	22	12.6

From Table III it is clear that in the majority of cases, both in sands and in soils, the bacteria are more efficient as producers of carbon dioxide when their numbers are not rising and less efficient as the numbers increase. That these results are not due to the fact that when low numbers of bacteria are present the culture is usually young and active and when high numbers are present it is staling, is shown in Table IV by considering the figures obtained when only the last five days of the culture's growth are considered.

Table IV.

Medium	++	+-	-+	--	$\chi^2$
Peptone	1	10	2	2	3.1
Soil extract	3	8	7	1	6.7
Minerals + glucose	0	7	3	0	—
All media	4	25	12	3	25.2

The obvious interpretation to place upon these results is that when the bacteria are reproducing, their energy is primarily devoted to building up fresh tissue, and that when reproduction is at a standstill, the energy is diverted and carbon dioxide is released in much larger quantities. In the case of young cultures, there is the anomaly that high efficiency for carbon dioxide production is correlated with active reproduction; apparently the bacteria of young cultures are able both to reproduce and to liberate carbon dioxide at the same time, whereas in the older cultures one or the other of the two processes predominates. These



contingency tables simply demonstrate that the efficiency varies according as to whether the numbers of bacteria in the cultures are increasing or decreasing, but they afford no information as to whether the efficiency is affected by the actual density of the population or as to whether it varies from medium to medium. If the efficiencies are grouped as in Table V, according to the number of bacteria present per gramme of

Table V.

*Bacterial efficiencies in gm. per 1000 million bacteria.*

		Numbers of bacteria in millions per gramme									
		No. of cases	0-200	No. of cases	200-400	No. of cases	400-600	No. of cases	600-800	No. of cases	Over 800
Soils											
Farmyard manured		2	·000245	2	·000140	7	·000126	3	·000098	33	·000039
Unmanured		12	·000149	9	·000053	1	·000055	2	·000049	2	·000049
NaNO <sub>3</sub>		24	·000365	10	·000058	5	·000062	1	·000029	—	—
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>		16	·000190	7	·000091	—	—	—	—	1	·000041
Sands											
Peptone		4	·000092	11	·000080	9	·000042	5	·000105	5	·000063
Soil extract		28	·000259	8	·000068	5	·000058	—	—	7	·000017
NaNO <sub>3</sub>		14	·000558	1	·000112	—	—	—	—	—	—
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>		19	·000790	—	—	—	—	—	—	—	—

Table VI.

*Amount of carbon dioxide in grammes given off from 400 gm. of different media by varying numbers of bacteria.*

		Numbers of bacteria in millions per gramme									
		No. of cases	0-200	No. of cases	200-400	No. of cases	400-600	No. of cases	600-800	No. of cases	Over 800
Soils											
Farmyard manured	Bacteria alone	—	—	—	—	—	—	1	·0228	32	·0216
	Bacteria and amoebae	—	—	—	—	1	·0256	1	·0404	31	·0240
Unmanured	Bacteria alone	11	·0072	9	·0056	1	·0100	1	·0128	2	·0068
	Bacteria and amoebae	11	·0068	7	·0068	4	·0104	—	—	2	·0440
NaNO <sub>3</sub>	Bacteria alone	13	·0072	8	·0052	4	·0072	1	·0092	—	—
	Bacteria and amoebae	15	·0068	8	·0072	—	—	—	—	2	·0076
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	Bacteria alone	16	·0104	4	·0112	—	—	—	—	1	·0160
	Bacteria and amoebae	17	·0100	7	·0112	1	·0072	—	—	1	·0152
Sands											
Peptone	Bacteria alone	4	·0056	11	·0084	9	·0088	5	·0272	5	·0252
	Bacteria and amoebae	14	·0076	9	·0136	6	·0160	2	·0112	2	·0300
Soil extract	Bacteria alone	27	·0052	8	·0072	5	·0116	—	—	3	·0104
	Bacteria and amoebae	20	·0044	11	·0172	3	·0080	1	·0068	8	·0064
NaNO <sub>3</sub>	Bacteria alone	20	·0040	1	·0096	—	—	—	—	—	—
	Bacteria and amoebae	20	·0088	—	—	—	—	—	—	—	—
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	Bacteria alone	20	·0036	—	—	—	—	—	—	—	—
	Bacteria and amoebae	19	·0060	—	—	—	—	—	—	—	—

medium, it is seen that the fewer bacteria there are present the more efficient each one becomes. Information as to the influence of the medium on the efficiency of the bacteria can be obtained by considering the actual amount of carbon dioxide given off by a constant volume of medium with the same numbers of bacteria. From Table VI, though the distribution is irregular, it would appear that although an increased population gives off an increased amount of carbon dioxide on any one medium, yet the bacteria produce roughly the same amount of carbon dioxide, irrespective of the medium. The different media are unable to support the same sized populations, since in the soils the unmanured and those with minerals rarely have a density of over 400 millions per gm., while the farmyard manured are almost entirely above 800 millions. This is also shown, though in a lesser degree, by the sand experiments, where peptone supports a larger population than the others.

#### *Effect of amoebae.*

In both sands and soils the presence of amoebae has the usual result of lowering the numbers of bacteria, but the effect upon carbon dioxide production is not so simple. In Table II the correlation between carbon dioxide production and bacterial numbers, when amoebae are present, is not so sharp as when bacterial cultures alone are considered; thus on soils there is no correlation in the unmanured and minerals, and in sand the same obtains for peptone and soil extract, but the ammonium sulphate and nitrate of soda sand cultures give a  $\chi^2$  over 4 and the farmyard manured soils one of 3.9. Such divergent results are only to be expected when it is remembered that the amount of carbon dioxide given off by amoebic respiration would often be sufficiently great to mask the direct effect from carbohydrate decomposition; and that this latter is conditioned by the continual fluctuations between the numbers of bacteria and amoebae. When the action of the amoebae on the amount of carbon dioxide produced is considered it is found that, in the case of sand cultures, amoebae decrease the amount of carbon dioxide given off when peptone is used, but increase the amount in the case of soil extract and of the mineral solutions with glucose (Figs. 1 and 2). When comparing each 24-hour period for the cultures, with or without amoebae, in soil extract, on 76 per cent. of the occasions more carbon dioxide was produced when amoebae were there; in the case of mineral salts and glucose there were 66 per cent. of times when the carbon dioxide was higher with amoebae, but in the case of peptone on 67 per cent. of the occasions the carbon dioxide was less in the presence of amoebae.

If the exceptions are examined the numbers are found to be even more significantly different than appears from the figures quoted. For instance in the soil extract cultures there are nine occasions when less carbon dioxide is produced where amoebae are present, but on five of these the bacteria are lower than is the case in the cultures of bacteria alone and the numbers of amoebae are negligible, and the same holds good for six out of the fourteen exceptions for the cultures with mineral salts and glucose. In the peptone experiments there are twelve cases where the amoebic cultures give more carbon dioxide than do the purely bacterial ones. In five of these the bacterial numbers are not significantly different in the two series of cultures, but the numbers of amoebae are very high, and the amoebic respiration might well bring up the output of carbon dioxide; in one case the bacteria were distinctly higher in the amoebic cultures and in two others the bacterial numbers were higher and the amoebae were also present in large numbers. Excluding therefore those which are explicable as shown above, the percentages of exceptions are reduced to 11, 20 and 10 per cent. in the case of soil extract, minerals and peptone respectively.

In soils the results are not so clear cut; in the case of the farmyard manured soil where the expectation is that less carbon dioxide would be produced in the presence of amoebae, as is the case with peptone in sand, in actual fact this only occurred on 47 per cent. of the occasions, and out of the seventeen exceptions only seven could be explained as being due to the numbers of bacteria being higher in the series with amoebae. The unmanured soil fell more into line with the sand treated with soil extract, in that the carbon dioxide produced was greater in 64 per cent. of the cases where amoebae were present, but of the ten exceptions only one could be due to the small numbers of both bacteria and amoebae in the amoebic cultures. The soils from the ammonium sulphate and nitrate of soda plots were again unsatisfactory, since only 50 per cent. of the cases gave higher carbon dioxide in the presence of amoebae, and of the fourteen exceptions only three could be accounted for by the low numbers of bacteria and amoebae in these cultures.

In view of the complex nature of soil compared with the simple inoculated sands it would be expected that the results would not be so clear cut; further work is therefore contemplated on soils together with experiments testing the amount of carbon dioxide which can be ascribed to amoebic and bacterial respiration as distinct from that produced by carbohydrate decomposition.

## SUMMARY.

Experiments are described on carbon dioxide production from soil and sand cultures containing a species of bacterium with and without amoebae. The following results were obtained:

1. Carbon dioxide production and bacterial numbers are correlated provided that amoebae are not present, or are present in very small numbers.

2. The bacteria are more efficient as producers of carbon dioxide when their numbers are not rising and less efficient when their numbers are increasing. This does not hold for young cultures. Also each bacterium becomes less efficient as the density of the population increases.

3. The amoebae cause a decrease in carbon dioxide production in sands containing peptone, but an increase in sands containing mineral salt solution with glucose or soil extract

## REFERENCES.

- (1) BARRATT, J. O. W. (1905). Die Kohlensäureproduktion von *Paramoecium aurelia*. *Zeitschr. f. Allgem. Physiol.* v, 66-72.
- (2) CUTLER, D. W. and BAL, D. V. (1926). Influence of Protozoa on the process of nitrogen fixation by *Azotobacter chroococcum*. *Ann. App. Biol.* XIII, 516-534.
- (3) CUTLER, D. W. and CRUMP, L. M. (1927). The Qualitative and Quantitative effects of food on the growth of a soil amoeba (*Hartmannella hyalina*). *Brit. Journ. Exper. Biol.* v, 155-165.
- (4) CUTLER, D. W., CRUMP, L. M. and SANDON, H. (1922). A Quantitative investigation of the Bacterial and Protozoan Population of the Soil. *Phil. Trans. Roy. Soc. B.* 211, 317-350.
- (5) FISHER, R. A. (1928). *Statistical Methods for Research Workers*. Edinburgh.
- (6) KONIG, J. and HASENBAUMER, J. (1920). Die Bedeutung neuer Bodenforschungen für die Landwirtschaft. *Landw. Jahrb.* LV, 185-252.
- (7) NELLER, J. R. (1920). The oxidising power of soil from limed and unlimed plots and its relation to other factors. *Soil Sci.* x, 29-39.
- (8) POTTER, R. S. and SNYDER, R. S. (1916). Carbon dioxide production in soils, and carbon and nitrogen changes in soils variously treated. *Iowa Agric. Stat. Res. Bull.* 39, 255-309.
- (9) PÜTTER, A. (1905). Die Atmung der Protozoen. *Zeitschr. f. Allg. Physiol.* v, 566-612.
- (10) RUSSELL, E. J. and APPLEYARD, A. (1915). The Composition of the soil atmosphere. *Journ. Agric. Sci.* VII, 1-48.
- (11) STOKLASA, J. and ERNEST, A. (1905). Ueber den Ursprung der Menge und die Bedeutung des Kohlendioxyds im Boden. *Centralbl. Bakt.* II, 14, 723-736.
- (12) STOKLASA, J. (1912). *Abderhald. Handb. biochem. Arbeitsmeth.* v, pt 2, 1925.
- (13) VAN SUGHTELEN, F. H. H. (1910). Ueber die Messung der Lebenstätigkeit der aerobiotischen Bakterien im Boden durch die Kohlensäureproduktion. *Centralbl. Bakt.* II, 26, 45-89.
- (14) WAKSMAN, S. A. (1927). *Principles of Soil Microbiology*. London.
- (15) WAKSMAN, S. A. and STARKEY, R. L. (1924). Microbiological analysis of Soils as an index of soil fertility. *Soil Sci.* XVII, 141-161.
- (16) WESTHUES, J. (1905). Die Kohlensäurebildung im Boden. *Inaug. Dissert. Münster i. W.*

(Received January 24th, 1929.)

## OBITUARY NOTICE

PROF. J. RITZEMA BOS

1850–1928

*Honorary Member of the Association of Economic Biologists.*

(With Plate XXI.)

J. RITZEMA BOS, one of the most distinguished pioneers in applied biological science, was born on July 27th, 1850, at Gröningen, where also he received his elementary and secondary education. At the University of his native town, besides studying in particular Zoology and Botany, he attended a course in Agriculture under H. C. van Hall. While still a student, he gained a University prize with a paper on "The Fauna of the Dutch Islands in the North Sea," and similar investigations formed the subject of his degree thesis, "Crustacea-hedriophthalmata of the Netherlands" in 1874. Before taking his doctorate, he had already (1869) been appointed teacher in Zoology at the Agricultural School in Gröningen.

Ritzema Bos' subsequent professional life may be briefly outlined. In 1871 he became a teacher in Zoology at the Agricultural Secondary School at Warffum (Friesland). Two years later we find him in a similar post at Wageningen and when, in 1876, a higher agricultural school was founded there, he was again appointed to teach his favourite subject. Nearly twenty years of research and teaching followed. The results were apparent when, in 1895, he was made head of the new Phytopathological laboratory—the "Willy Commelin Scholten"—in Amsterdam. This was a strenuous period in Ritzema Bos' life, as he was also assistant-professor in the University and continued to discharge his duties at Wageningen.

It was not, however, till four years later that Prof. Ritzema Bos' ideas of the rôle of Applied Biology in Agriculture, Forestry and Horticulture began to have full effect with the founding in Amsterdam of a national Phytopathological Service—an institute designed not merely to diagnose and prescribe, but, above all, to see that the advice given is efficiently carried out. Such a service was fittingly enough inaugurated in a land which, for over three centuries, had been noted for its intensive cultivation. At the present day, *e.g.* two heavy crops of cabbage per field

is the rule, and the average yield of potatoes is 241 hectolitres per hectare, while exceptionally 329 hectolitres may be raised. Of this new service Ritzema Bos became head (1895)—his whole career since 1869 fitted him unquestionably for the post—and his impress on it has been deep. At present the service, with a personnel of sixty official members, in conjunction with three hundred voluntary co-operators (for the most part prominent local agriculturists or horticulturists holding the diploma of a recognised school) carries out a vast and varied campaign against disease in plants. The work is divided into several sections, embracing research into cases submitted (about two hundred per month) and the giving of advice; propaganda—with two branches dealing with diseases in horticulture and agriculture respectively; ornithology—concerned with aviculture in woods and orchards. (An interesting and original departure, *e.g.* nesting boxes and breeding holes are multiplied and the results tabulated; effects of egg-gathering on gulls and plovers studied; general investigation of bird food and special research carried out on such species as the rook, starling and plover.) There are also sections (*a*) for museums, collections and exhibitions, (*b*) inspection and the enforcing of legislation, (*c*) administration and statistics.

In 1906 Ritzema Bos became Head of the newly founded Institute for Plant Diseases at Wageningen, and lecturer in Phytopathology in the higher agricultural school. In 1918 this school was raised to the status of a University of Agriculture and Dr Ritzema Bos became a professor. When, in 1919, the Phytopathological Service was separated from the Phytopathological Institute, he retired from the headship of the former and next year, at the age of seventy, gave up official work. After his retirement, however, he still acted as adviser to the Phytopathological Service.

Although Dr Ritzema Bos' interests lay, at first, in animal physiology, he soon devoted himself entirely to research in phytopathology, and there especially to helminthology and economic entomology.

In 1891 he founded the Nederlandsche Phytopathologische Vereeniging in conjunction with Prof. Hugo de Vries. He was president of this Society till his death. From 1895 he edited the *Tijdschrift voor Plantenziekten*. With the late F. B. Löhnis and the late Dr P. van Hoek and L. A. Springer, he edited the *Geïllustreerde land- en tuinbouwbibliotheek* (Illustrated Agricultural and Horticultural Library).

Amongst his principal contributions to biological literature are the following: "L'Anguille de la Tige (*Tylenchus devastatrix* Kühn), et les maladies des plantes dues à ce Nématode," *Archives Teyler*, Harlem,



PROFESSOR J. RITZEMA BOS





1888-92, Sér. II, Tome III, and, in the same series (Tome II, 2, 1885) appeared his monograph, "La Mouche du Narcisse (*Merodon equestris* F.), ses métamorphoses, ses mœurs, les dégâts causés par les larves et les moyens proposés pour la détruire." His well-known *Zoologie für Landwirte* had a great vogue on the continent, attaining to a seventh edition in 1923. Translated into English by R. A. Davis under the title *Agricultural Zoology* (1894), the book was welcomed and attained a second edition (1900). Another major work was *Tierische Schädlinge und Nutzlinge*, 1891. In the Illustrated Agricultural and Horticultural Library, above referred to, Dr Ritzema Bos contributed longer articles on the anatomy and physiology of domestic animals; diseases and injuries of fruit trees; diseases of crops, etc. For his students also, he produced text-books on general and agricultural zoology.

From 1870 onwards Ritzema Bos published very many notes, observations and shorter articles which will be found mainly in the following serials: *Tijdschrift voor Plantenziekten*; *Tijdschrift voor Entomologie*; *Archives Teyler*, Harlem; *Die landwirtschaftlichen versuchstationen*; *Zeitschrift für Pflanzenkrankheiten*; *Biologisches Zentralblatt*; *Zeitschrift für Bacteriologie und Parasitenkunde*.

Beyond his native land, Dr Ritzema Bos' great experience and special knowledge were widely recognised. He was one of our oldest honorary members, and had received a similar distinction from the American Association of Economic Entomologists, and the Deutsche Gesellschaft für Angewandte Entomologie. In 1899 he was honoured with a knighthood (Knight of the Dutch Lion) for his services to the State.

In the course of a long professional career, he travelled extensively, sometimes for the purpose of research, as *e.g.* when in 1899 he studied the San José scale, or again when, as an official delegate, he returned to the United States (1921) to discuss the conditions of the import of Dutch bulbs, shrubs and trees. Ritzema Bos' position as a writer and organiser is an assured one. A man of rare and practical vision he laid the foundations of applied biology in Holland with a firm hand. By his students, too, he will always be remembered for his unselfish helpfulness and the constant inspiration of his indefatigable work.

(I am greatly indebted to my friend, Dr J. Betrem, Entomological Laboratory, Wageningen, for many of the details of this short notice—particularly as regards Dr Ritzema Bos' early life. The accompanying photograph, taken in May 1924, is by Dr Roepke.)

J. WATERSTON.

## REVIEWS

*Plant Diseases.* By F. T. BROOKS. Oxford University Press, 1928.  
Pp. vi + 386. 62 Illustrations. Price 21s. net.

Since the publication in 1910 of Massee's *Diseases of Cultivated Plants and Trees* there has appeared in this country no general account of the diseases of British crop plants. "The present book endeavours to supply this deficiency. In it the author has also attempted to include an outline of our present knowledge of important plant diseases in other parts of the Empire and in other countries of the world." This wider field has of course been covered by numerous treatises published in America, Germany and elsewhere during recent years. The scope of the present work is briefly as follows.

Chapter I is a general introduction to the subject, dealing shortly with economic losses, etiology, symptoms and spread of disease, the influence of environmental conditions and disease control. Chapter II is a summary treatment of non-parasitic diseases. Chapters III-VI are devoted respectively to diseases caused by Viruses, Bacteria, Actinomycetes and Myxomycetes. Chapter VII is introductory to the main part of the book and contains a brief general account of the fungi with a synoptic scheme of classification. Chapters VIII-XX are given to diseases caused by fungi. Beginning with Phycomycetes, the author works through the Ascomycetes, Basidiomycetes and Deuteromycetes. In turn each group is briefly described, then the alliances and the genera within the alliances and finally the diseases caused by particular species. The scientific name of the fungus is followed by the common name of the disease recommended by the British Mycological Society, a synopsis of the fungus and an account of the host plants, symptoms, distribution and treatment of the disease. In chapter XXI the algal disease of tea is described and the last chapter contains a brief account of some common fungicides and is followed by an index. To each chapter is appended a selected list of references.

The sixty-two illustrations deserve comment, favourable in that they are all original, unfavourable in that some of them are of mediocre quality. Further it is not easy to understand the rationale of the author's choice or apportioning of illustrations for they seem to bear little relation to the comparative importance of the several diseases or groups of parasites.

Whilst reading the volume one is not infrequently arrested by points on which opinions might diverge but these usually concern detailed matters and merely emphasise the "up-to-dateness" and general excellence of the work and the relatively enormous amount of matter the author has compacted within it.

The criticism one might make of this book is more fundamental, namely, that it presents what many pathologists would consider a wrong balance of values, and as the book is probably destined to become the accepted English text this is a matter of real concern. The work is entitled *Plant Diseases*, with no qualifying sub-title, and the preface states that "The author has had a good deal of experience in training men to serve as Plant Pathologists at home and abroad, and his book is designed particularly to assist such students and others who are carrying out investigations on Plant Diseases. It is hoped also that the book will be useful to the general botanist, to students of Agriculture, Horticulture, and Forestry, and to those cultivators of the soil who take an enlightened interest in the crops they grow." The work therefore represents the author's matured judgment of the comparative importance of the several regions of plant pathology and of the different diseases of Empire crops. The minor criticism may be illustrated by a single example. It is easy to sympathise with Mr Brooks' tendency to overflow on silver-leaf disease, but it hardly seems justifiable to devote six pages to this one malady and only twelve pages to either the specific virus diseases or the bacterial diseases. The larger criticism however involves the orientation of the author's view-point towards the science of plant pathology. Diseases

of plants are usually divided into two groups—"non-parasitic diseases" and "parasitic diseases." Mr Brooks accepts this division but, merely stating that "In this book it is not possible to give a full account of non-parasitic diseases, and only a few of the commoner maladies of this kind will be discussed," he waives them aside in eight pages with no illustrations. It might be thought equally impossible to give a full account of parasitic diseases yet these receive 340 pages and 62 illustrations. It is almost as if the functional diseases were practically omitted from a medical text-book, their exclusion being justified by the author on the ground that it was not possible to give a full account of them, or as if in the present work Mr Brooks had reversed his procedure and had discussed the non-parasitic diseases in 340 pages and dismissed in eight pages the diseases caused by viruses, bacteria and fungi. The difference in view-point may be appreciated if one compares the present volume with standard works which have recently appeared in Germany, France and the United States of America, for example Sorauer's *Handbuch der Pflanzenkrankheiten*, Delacroix and Maublanc's *Maladies des Plantes Cultivées* and Heald's *Manual of Plant Diseases*.

To justify the title of "Plant Diseases" and give a right perspective to those for whom the author states that he has written the book, one may hope that he will regard this as volume one and produce an equally useful second volume in which the non-parasitic diseases receive due consideration.

The book is dedicated to Marshall Ward, of whom a fine portrait serves as frontispiece, and it is well produced but misprints have been noted on pages 50, 205, 246, 361 and 371, and some word such as "only" seems to have been omitted from line 3 on page 263.

As a handy compendium of the fungus diseases of plants the work is one of the most useful additions to the literature of plant pathology that has been published for sometime and it will be found indispensable by all British students and practitioners of the science.

WILLIAM B. BRIERLEY.

*Laboratory Manual of General Microbiology: with special reference to the micro-organisms of the Soil.* By E. B. FRED and S. A. WAKSMAN. McGraw-Hill Publishing Co., Ltd., 1928. Pp. v + 145. 19 Figs. Price 10s. net.

In America particularly, and due in no little part to the activities of the two authors, a scientific synthesis known as "General Microbiology" is rapidly developing. It is concerned with the lives and activities of the simpler algae, lower fungi, bacteria and protozoa as these occur in diseased states, industrial processes, soil, sewage and other economic environments. The American synthesis received general statement in the valuable compilation edited by C. E. Marshall some years ago but since then considerable advances have been made and particular aspects, such as soil microbiology or the microbiology of disease, have become prominent scientific issues. The general science is taking an important place in the curriculum of agricultural colleges, technical institutes, etc. and is gathering about itself a considerable special literature. The present book belongs to this and has been designed specially for students of soil-biology. It is divided into four parts. The first contains general directions for the making of culture media and details of one hundred and eleven different media for all sorts of micro-organisms. Part II is devoted to methods of staining and is not quite so good as it might have been. Part III is a very useful account of qualitative and quantitative methods of analysis and is written simply and practically. Part IV consists of 58 laboratory exercises covering generally the study of micro-organisms in the soil. It also contains a classified list of the more important books of reference and the addresses of laboratories where pure cultures may be obtained.

Throughout, special attention is paid to the physiology of the micro-organisms, more especially perhaps to their fermentation processes, in an attempt to portray not only their qualitative but also their quantitative relationships. No attempt is made to mention all the biological or chemical methods employed in microbiological

studies and it is in the selection of one method rather than another that scope is, perhaps, afforded for criticism. On the whole the authors seem to have been extremely judicious in their selection which must have been a difficult task.

The book is well produced and strongly bound, a quality very desirable but often overlooked in a volume whose fate it is to lie about on working benches. It will be found very handy in all laboratories where micro-organisms are studied.

WILLIAM B. BRIERLEY.

*The Principles of Applied Zoology.* By ROBERT A. WARDLE. Longmans, Green & Co., 1929. Pp. xii + 427. 55 Illustrations. Price 21s. net.

This book attempts to embrace in one compass the whole of the diverse aspects of zoology in relation to man and his operations. We cannot recollect a similar kind of book covering so wide a field—in fact so wide is the field that it is usually catered for in a number of separate treatises. In attempting this task Prof. Wardle has at least shown how numerous are the phases of human activity which require expert zoological knowledge. At the same time he has compressed a large amount of information in a relatively small compass.

Part I, which consists of twelve chapters, deals with medical and veterinary zoology. Four chapters are concerned with Protozoa, their structure, classification and distribution, followed by accounts of the enteric and haematophilous forms. A similar number of chapters are given over to Helminthes and likewise to Arthropoda. Part II also comprises twelve chapters and is concerned with agricultural and horticultural zoology. The first chapter discusses in general terms the fauna of the soil. Then follow six chapters on insect pests and a chapter each on Vermin Repression, Bird Encouragement, Animal Domestication, Types and Breeds of Farm Animals and Livestock Breeding. Part III is concerned with animal industries including bee-keeping, silk and lac culture, fisheries, whaling and sealing, fur-bearing animals and the trades concerned and animal conservation. There are ten chapters on these subjects and they extend over less than 100 pages. Part IV of the book is composed of a classified bibliography and an index.

Viewing the book as a whole we can say definitely that Prof. Wardle has surveyed his subject well. The real trouble is that he has set himself the task of taking cognisance of the whole realm of applied zoology within a compass of about 400 rather small pages. He obviously has had to cut his cloth according to the amount available and too much must not be expected for that reason. He has, however, succeeded in providing an extraordinarily wide survey of his subject, based upon an extensive acquaintance with its literature. Since his volume bears the title of "Principles" it is perhaps a little anomalous to find that it is much stronger in facts and that principles rather fade into the background. In some of the chapters, for example, it is not easy for the reader to gather where the zoologist comes in and how he can assist the practical man. One of the greatest contributions of zoology to practice is the unravelling of the laws of heredity. Man is thus provided with the means by which he is enabled to improve animal breeding, whether the animals be cattle or silkworms. In apiculture we need a method of scientifically controlled mating of the queen bee, and, with the possibilities of artificial insemination, that desideratum may already be in sight. With noxious insects the application of insecticides has long held the field, but we are beginning to realise the importance of studying those factors which conduce to infestations. A notable advance may well come from zoological studies in this direction combined with help from other sciences. Of lac culture India has a virtual monopoly, and one of the obstacles to improvement is the difficulty of eliminating parasites—and here the skilled zoologist is essential. With fisheries the big zoological problem is the study of food-fishes to a degree of exactness, as regards their behaviour and conditions of life, that will enable us to conserve our diminishing supplies: and the same applies to whaling. These are but a few examples of how the applied zoologist, utilising advances in pure science, can make himself indispensable.

We think that applied zoologists will welcome this volume as a comprehensive

outline of their subject. Its freedom from errors and the clear method of presentation commend it to the student, while the useful bibliography at the end guides the reader to abundant sources of further information.

A. D. IMMS.

*Agricultural Entomology.* By D. H. ROBINSON, B.Sc. and S. G. JARY, B.A.  
London: Duckworth, 1929. Pp. vii + 314. 149 Text-figures. Price 15s. net.

For a number of years past there has been a very evident need for a reliable up-to-date text-book of agricultural entomology and even to this day John Curtis' *Farm Insects* is still, in spite of its antiquity, the most exhaustive volume we possess. A second "Curtis" is badly required, but it demands exceptional qualifications on the part of anyone who is sufficiently public spirited to embark on its production. He must be a man endowed with breadth of outlook on both the scientific and applied aspects of his subject and, at the same time, capable of utilising to the best advantage the mass of valuable information that is yearly accumulating in continental journals and text-books.

The volume before us is of a much more elementary character than the one just envisaged. It is a students' handbook and one necessarily confined, owing to limits of space and cost, to a briefer method of treatment. The first part is devoted to an outline of the general structure, life-histories and classification of insects. The second part deals with the various insects of importance to agriculture under their respective orders, and briefly describes preventive or remedial measures for combating them. At the end of this section are two chapters dealing respectively with the principles of insect control and with the composition and application of insecticides. A number of animals, other than insects, which also concern the agriculturist are discussed in a series of short appendices. The book concludes with hints on entomological technique, a short list of literature and an index.

Of the two authors Mr D. H. Robinson is Head of the Biology Department of the Harper Adams Agricultural College and Mr S. G. Jary is an Advisory Entomologist for the Southern Province under the Ministry of Agriculture scheme. Viewed as a whole the book they have written is up to date and should meet the demand it is evidently intended to cater for. It gives the essential information the student requires and, if supplemented by adequate laboratory and field training, the candidate should then be sufficiently well equipped to fulfil the requirements in entomology for an agricultural degree or diploma. Its clear method of expression, excellent type and good text-figures make it an attractive volume. If the practical farmer or grower can be induced to read it, he will find himself tolerably well informed of the various pests he has to contend with; he will also learn about some of the control measures which he is, as a rule, much more interested in. As many garden pests are also included it should further appeal to a proportion of the general public.

It is usually considered to be a reviewer's function to point out errors and omissions, even if it is only to prove that he has actually read the book before him, but they often tend to prejudice a book to a disproportionate degree. It is necessary, however, to mention that there are slight errors on pp. 42 and 43; the legends of Figs. 70 and 72 have been inadvertently transposed; on p. 92 Membracidae should read Cercopidae; and in the account of the Diptera (p. 193) the authors have misunderstood the significance of the frontal lunule. Such points will doubtless be corrected in the event of a new edition being called for. At the same time the authors would be well advised to give more definite information with reference to the control of the apple Capsid (p. 93), Millipedes (p. 285) and some other pests. With regard to Leather Jackets (p. 200) it appears that they are unaware that the Protection of Animals Act of 1911 was amended in 1927 so as to allow under "reasonable precautions" of the application of such methods as poison baits which are a valuable control measure.

A. D. IMMS.

## PROCEEDINGS OF THE ASSOCIATION OF ECONOMIC BIOLOGISTS

ORDINARY MEETING of the Association held at 2.30 p.m. on March 15th in the Imperial College of Science and Technology, London. The President, Dr E. J. Butler, C.I.E., F.R.S., in the Chair.

- I. "Some Agricultural Problems in Australia" by Sir JOHN RUSSELL, F.R.S.
- II. "The Commonwealth Council of Science and Industry in its relation to Agriculture" by F. L. McDOUGALL, Esq., C.M.G.

Meeting of the Association on May 10th at the Docks of the Port of London Authority. London and St Catherine's Docks—Cacao, Dried Fruit, Spices, Ivory, Wool, Essential Oils and Wines. Royal Victoria and Albert and King George V Docks—Methods of handling and storage.

Field Meeting of the Association at Cambridge on Friday, June 21st, and Saturday, June 22nd. Visits to University Farm, Potato Virus Research Station, Low Temperature Research Station and Wicken Fen. Association Dinner in Christ's College.

## INTERNATIONAL CONFERENCE FOR PHYTOPATHOLOGY AND ECONOMIC ENTOMOLOGY—HOLLAND, 1923.

### SECOND NOTICE ON ERIKSSON PRIZES.

The Committee beg to announce that two prizes are hereby offered for the two best Memoirs, giving an account of new and original work on the two following subjects respectively:

- (1) Investigations on Rust (Uredineae) Diseases of Cereals (Wheat, Oats, Barley or Rye).
- (2) Investigations on the rôle played by insects or other invertebrates in the transmission or initiation of Virus Disease in Plants.

The value of each prize will be 1000 Swedish crowns.

Competitors may be of any nationality.

Three typewritten copies of each Memoir must be submitted. They may be written in any one of the three languages, English, French or German.

Memoirs must reach the Secretary on or before May 1st, 1930.

The author's name must not appear on the Memoir itself, but each Memoir must be marked with a pseudonym or a motto and the full name and address of the author must accompany the Memoir, being enclosed in a sealed envelope bearing on its outside the same pseudonym or motto as is given on the Memoir.

The adjudication of the rust prize will rest with a Jury, consisting of Prof. Dr Jacob Erikson, Prof. Dr E. C. Stakman and Prof. Ét. Foëx.

The adjudication of the virus prize will rest with a Jury, consisting of Prof. Dr H. M. Quanjér, Dr A. D. Imms and Dr L. O. Kunkel.

The decisions of these Juries will be final, and will be announced at the Fifth International Botanical Congress, to be held in Cambridge (England) from August 16th to 30th, 1930. The copyright of the prize Memoirs will become the property of the Committee, who will endeavour to secure publication of them in a suitable existing periodical or, failing that, procure publication in some other way. Other Memoirs will be returned to their respective authors.

The Committee reserve the right to withhold the prizes should none of the Memoirs submitted be deemed of sufficient merit by the respective Juries.

Further particulars, if required, may be obtained on application to the Secretary.

For the International Committee for Phyto-  
pathology and Economic Entomology

(Signed) T. A. C. SCHOEVEERS, *Secretary*,  
Nassauweg 28, Wageningen. Holland.





# MANGANESE AS AN ESSENTIAL ELEMENT FOR PLANT GROWTH

BY GEOFFREY SAMUEL (Plant Pathologist)

AND C. S. PIPER (Chemist).

(*Waite Agricultural Research Institute University of Adelaide.*)

(With Plates XXII-XXIV and 2 Text-figures.)

## CONTENTS.

	PAGE
I. INTRODUCTION . . . . .	494
Historical . . . . .	495
Naturally occurring manganese deficiency diseases . . . . .	497
II. EXPERIMENTAL WORK . . . . .	498
A. Plants tested in water-culture for the essential nature of manganese . . . . .	498
Symptoms of manganese deficiency . . . . .	499
B. Experiments with oats . . . . .	503
(a) The non-replacability of manganese with other elements . . . . .	503
(b) The effect of different concentrations of manganese in the nutrient solution . . . . .	504
(c) The effect of removing manganese at different stages of growth . . . . .	505
(d) Comparison of oats and rye as to manganese requirement . . . . .	506
C. Chemical determinations of the manganese content of oats grown under different conditions . . . . .	507
(a) Variation in manganese content with growth (oats and barley) . . . . .	507
(b) The variability in manganese content of individual oat plants . . . . .	509
(c) The minimum amount of manganese found in healthy oat plants at the flowering stage . . . . .	511
D. Experimental work relating to factors possibly influencing the appearance of manganese deficiency in oats in the field . . . . .	513
(a) The effect of excess of calcium ions in water-culture . . . . .	513
(b) The effect of excess of nitrate ions in water-culture . . . . .	514
(c) The effect of the presence of organic compounds . . . . .	514
(d) Chemical determinations of manganese in plants from manganese deficient soils as compared with that in plants from normal soils . . . . .	515
III. DISCUSSION . . . . .	516
IV. SUMMARY . . . . .	519
V. APPENDICES:	
I. Water-culture methods . . . . .	520
II. Method for the chemical determination of manganese in plants . . . . .	522
REFERENCES . . . . .	523
EXPLANATION OF PLATES . . . . .	524
<b>Ann. Biol. XVI</b>	<b>33</b>

## I. INTRODUCTION.

IN a previous paper (Samuel and Piper, 1928) it was shown that the Grey Speck disease of oats, which occurs on certain types of soil, is a manganese deficiency disease. A certain amount of evidence was also given that it is rather a lack of *available* manganese in the soil than actual manganese deficiency which is responsible. Soils which do not appear to have sufficient available manganese for the growth of oats (and to a less extent of barley and wheat), nevertheless support an apparently normal growth of pasture plants and weeds. The question is therefore raised as to the necessity of manganese for such pasture plants; and, if it is necessary, whether it is that they require much smaller quantities for normal development than do the cereals, or whether they are able to absorb more of what manganese is present than can the cereals. (It may be noted here that rye is an exception among the cereals, and will grow well on land where oats suffer severely from manganese deficiency.)

Of recent years, mainly owing to the work of McHargue (1922, 1926), it has come to be recognised that manganese is an essential element for plant growth. McHargue showed, by sand and water culture experiments, that a number of plants could not be grown to the fruiting stage in the absence of manganese. With wheat in water-cultures he obtained an increase of 135 per cent. in dry weight in the plants supplied with manganese as compared with those deprived of this element, and with peas an increase of 67 per cent.

In the present investigation it has been found that when careful precautions are taken to exclude all traces of manganese, the increases in dry weight in different plants to be expected from the addition of manganese range from 300 to over 5000 per cent. Except in the case of rye, the increases for the cereals and grasses used all ranged from 1000 to 5000 per cent.

Such striking results as these were not obtained by McHargue, and it would appear probable that traces of manganese must still have been available to the plants in his experiments which he considered to be growing in solutions free from manganese. That such results would probably be obtained if extra care was taken in the purification of chemicals and exclusion of manganese was forecasted, however, by Sommer and Lipman (1926), who obtained similar results in their experiments on the necessity of zinc and boron for the growth of higher plants.

*Historical.*

Published work on the relation of manganese to plant growth is very voluminous. A review of much of the earlier work is given by Brenchley (1927).

Bertrand, led on to the subject from his work on the relation of manganese to laccase, has been insisting for nearly thirty years on the essential nature of manganese in the plant economy. It was the universal presence of manganese in plant ash, and its beneficial effect as a fertiliser, as well as his experiments on its relation to oxidases, which led him to this opinion. He did not support it with definite culture experiments with precautions to exclude manganese, except in the case of the fungus *Aspergillus niger*, which he showed would not form conidia in the absence of this element.

The work of Bertrand, together with that of investigators in Japan (Loew, Aso, Sawa and others) on the stimulating action of manganese salts on rice and other plants, started a vogue for so-called "catalytic" fertilisers, among which manganese was perhaps considered the most important. This was reflected in the publication of the results of numerous empirical fertiliser tests on all kinds of agricultural crops, using the salts of manganese and other rarer elements.

Many of these tests showed appreciable increases in crop yield as a result of the use of fertilisers containing manganese, but others provided only doubtful or negative evidence of their value. Very few of these publications contained any information whatever upon either the manganese content or the reaction of the soil upon which the experiments were tried.

The earlier water or sand-culture work was concerned more with the toxicity of manganese, which may be evident in dilutions as low as 1 : 1,000,000, than with its essential nature, although a "stimulant" action was noted at high dilutions by Aso as early as 1902. It was not until 1914 that Mazé began to investigate the problem of the necessity of traces of the rarer elements by means of water-cultures with carefully purified salts. He demonstrated that manganese, as well as a number of other rarer elements, was necessary for the growth of maize. McHargue (1922) later proved by sand and water-culture experiments that manganese was necessary for the growth of a considerable number of plants. Schreiner and Dawson (1927) and Miller (1928) have confirmed this in pot experiments with tomatoes and certain other plants. Bishop (1928) also found manganese necessary in sand cultures with maize, peas, beans

## 496 *Manganese as an Essential Element for Plant Growth*

and radishes. The necessity of manganese for normal growth is now recognised to such an extent that it is included as a matter of course in a complete nutrient solution for water-culture work such as that of Sommer and Lipman (1926) and Sommer (1928).

The *symptoms* developed by plants suffering from manganese deficiency have been described by all the above authors as a chlorosis. McHargue (1922) states, "the first effect to be noted in the growth of plants from which manganese has been withheld is a lack in the development of chlorophyll in the newly formed tissues or in the growing parts of the plant. This condition increases with time, and finally results in the tips of the branches dying back and a cessation of further growth of any consequence in the plant."

Schreiner and Dawson (1927), describing the symptoms of manganese deficiency in tomatoes, say, "the chlorosis manifests itself at first as a lightening of the green colour, turning to yellow, in the leaf areas farthest from the major veins. As the condition progresses the yellow becomes more marked and extensive, the veins still remaining green, giving a characteristic mottled appearance to the leaf. Eventually the foliage may become completely yellow; and in many cases, especially on the untreated soil, a necrosis sets in, appearing at first as tiny brown pin-points centring in the yellow areas farthest from the veins and expanding to larger dead areas indicating complete breakdown of the tissues."

Miller (1928) also describes the effect as a "chlorosis which is apparently quite characteristic, being chiefly recognised by the fact that in most plants the yellow colour is located in areas away from the veins, thus producing a mottled appearance, or in the case of grasses, a striped effect."

It would appear from these quotations that the symptoms of manganese deficiency are not those of a normal chlorosis in the usual sense of the word, such as results from iron deficiency. This is a point which does not seem to have been sufficiently emphasised in the past, and it is further discussed below.

With regard to the function of manganese in plants, although a number of theories have been advanced, none of them is supported by sufficient experimental work to make any one of them conclusive. Bertrand considered that manganese was an essential part of the oxidase system of plants. McHargue (1922) claimed that manganese performs an important function in carbon assimilation and the synthesis of

chlorophyll, and later (1924) drew attention to an apparent correlation between the occurrence of manganese and vitamins.

*Naturally occurring manganese deficiency diseases.*

For a long time the Grey Speck disease of oats seems to have been the only manganese deficiency disease occurring in the field for which it was recognised that an application of a soluble manganese salt was the best cure (Riehm, 1917). At the same time it does not seem to have been recognised that the symptoms of the Grey Speck disease are essentially the symptoms of manganese deficiency until this was proved by the present writers (1928). It was known that the trouble appeared on certain types of soil, from nearly neutral to fairly strongly alkaline in reaction, but very different theories were advanced as to the cause of the trouble. Hudig (1923) considered that small amounts of certain organic substances, in conjunction with the alkaline reaction, were responsible. Arrhenius (1923 and 1924) considered that the disease was always associated with an excess of calcium ions in the soil solution. Hiltner (1924) believed that the beneficial effects of manganese were due to an indirect effect on the carbonic acid assimilation of the plants. He formulated a "Carbonic-acid mineral-substance Law," and advanced the proposition that under conditions unfavourable for adequate carbon assimilation the stimulatory action of manganese enables the plants to assimilate their food supply and make full use of the nutritive elements supplied by the soil.

The next naturally occurring manganese deficiency disease for which it was recognised that an application of a manganese salt was a cure was the chlorosis of spinach described by McLean and Gilbert (1925). At this time, however, the importance of manganese as a plant nutrient was being emphasised by McHargue, and it was realised from the commencement that the spinach trouble was definitely a result of manganese deficiency (Gilbert and McLean, 1928).

Later Schreiner and Dawson (1927) showed that failure of tomatoes in certain highly calcareous glade soils could be corrected by an application of sulphate of manganese.

Willis (1928) showed that oats and soy beans suffered from manganese deficiency on some coastal plain soils of North Carolina.

Finally, Lee and McHargue (1928) have demonstrated that Pahala blight of sugar cane is a manganese deficiency disease, giving chemical analyses as well as demonstrating that the application of manganous sulphate would cure the trouble.

## II. EXPERIMENTAL WORK.

The experimental work aimed at investigating the essential nature of manganese for plant growth by means of water-cultures with greater precautions than have been observed hitherto; and at determining factors influencing the absorption of manganese, by means of water-cultures and chemical determinations of the manganese present in experimental and in field plants.

The special precautions observed in the water-culture work, including preparation of a manganese-free iron salt, and paraffining of all glass surfaces in contact with nutrient solutions, are detailed in Appendix I.

## A. PLANTS TESTED IN WATER-CULTURE FOR THE ESSENTIAL NATURE OF MANGANESE.

Table I gives a list of twenty plants which were grown in water-culture to determine whether manganese was essential for their normal development.

Table I.

*Showing the dry weights of plants grown in water-cultures with manganese and without manganese.*

Dry weight in gm.			
		With manganese 1 : 1,000,000	Without manganese
Wheat: Federation	...	*	*
Sepoy	...	150	14
Late Gluyas	...	149	6
Oats: Algerian	...	185	8
Lachlan	...	*	*
Mortgage Lifter	...	*	*
Imbros Island	...	*	*
Barley: Cape	...	101	6
Rye	...	86	27
<i>Danthonia penicillata</i>	...	47	0
<i>Lolium subulatum</i>	...	131	19
<i>Phalaris bulbosa</i>	...	114	0
<i>Bromus unioloides</i>	...	109	4
Peas	...	46	11
Broad bean	...	87	26
Lucerne	...	20†	7
<i>Medicago denticulata</i>	...	17†	4
<i>Trifolium subterraneum</i>	...	48†	5
Tomato: Dwarf Red	...	—	—
Maize	...	158	29

\* Varieties not carried on after manganese deficiency symptoms established with certainty.

† Changed after six weeks to Mn 1 : 5,000,000 solution, since Mn 1 : 1,000,000 appeared toxic.

Four culture jars of each variety, each containing six seedlings (except in the case of broad bean, where there were only two seedlings), were prepared with manganese-free culture solution. To two of these jars 10 c.c. of 128 per cent. pure manganese sulphate ( $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ ) were added, sufficient to make the concentration of manganese in the solution one part in a million.

Boric acid (1 : 2,500,000) was added to all jars containing leguminous plants.

Losses due to transpiration were replaced with distilled water, and the solutions were changed twice during the growing period.

In many cases the seedlings in the two manganese-free jars were either dead or almost dead before the first change of solution, about ten weeks after commencement. Those in the solutions containing manganese grew vigorously and healthily at all times. Occasionally plants became somewhat yellowish as if needing more iron, and in these cases an extra half dose of ferric citrate was given to all four jars of a series, even if only one jar was showing the need of iron.

The cultures were kept going until the plants in jars containing manganese had passed the flowering stage, and in some cases had formed ripe seed (Plate XXIII, fig. 1). They were then harvested and the dry weights determined. (Varieties of oats other than Algerian were not carried on after pronounced symptoms of manganese deficiency had manifested themselves on all the seedlings in manganese-free solutions.)

The effect of withholding manganese from the plants can be well seen in the figures in Plates XXII and XXIII. None of the plants tested has been able to develop beyond the seedling stage in the absence of this element. This fact is reflected in the comparative dry weights of the cultures (Table I) which show increases ranging from 300 to over 5000 per cent., due to the addition of one part of manganese (as sulphate) to a million of culture solution. There is little doubt that the lower figures would also have been considerably increased if the best culture conditions had been known from the start. The clovers, for example, did not grow well in the jars containing manganese 1 : 1,000,000 and boric acid 1 : 2,500,000, but when the concentrations of each were reduced to 1 : 5,000,000 they grew ahead normally.

#### *Symptoms of manganese deficiency.*

In general it may be said that all plants germinated and grew healthily for a few weeks, presumably with the aid of the manganese stored in the seed. The amount of healthy growth made in manganese-free solutions

## 500 *Manganese as an Essential Element for Plant Growth*

was in some relation to the size of the seed. Peas and beans grew to nine inches or more in height before cessation of growth, whereas cereals usually showed the deficiency about the time of tillering, when they were from three to five inches high, and some grasses with small seeds scarcely grew at all in the manganese-free solutions.

In all cases the onset of manganese deficiency symptoms was comparatively sudden.

*Peas.* With peas, for example, the growth in the manganese-free solutions appeared just as healthy and vigorous as that in the solutions containing manganese for 38 days from the date of germination. Growth

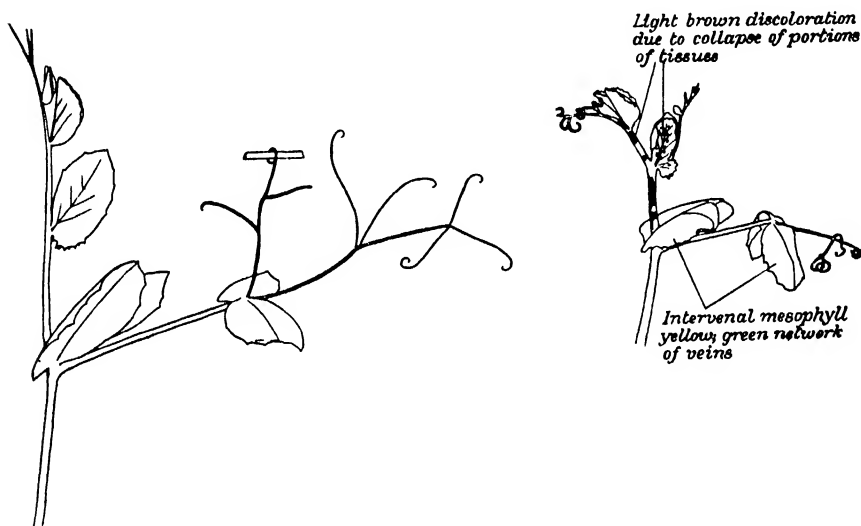


Fig. 1. The growing points of two plants of Brunswick White Peas; that on the left from water-culture containing nutrient solution plus manganese 1 : 1,000,000, and that on the right from water-culture containing nutrient solution free from manganese.

of the plants in the manganese-free solutions then ceased, young tendrils and the youngest internodes at the top of the stem acquired a brownish discoloration, at first superficial, and the youngest leaves failed to expand, becoming yellowish with small discoloured areas between the veins where the mesophyll had collapsed. Slightly older, but not fully expanded, leaves acquired a characteristic mottled appearance due to the mesophyll between the small veins becoming yellow, the veins themselves remaining green and forming a fine green network over the leaf with meshes of yellow about 1 mm. in diameter. All the lower fully formed leaves retained their normal green colour. The changes at the



growing point were pronounced within two or three days after the symptoms were first noticeable, and the plants in the solutions containing manganese offered a striking contrast, being already several inches higher and with normal healthy green growing tips (Fig. 1). No further growth took place in the solutions free from manganese, the growing tip and the youngest leaves being completely dead in a fortnight. The lower part of the plants remained alive for months. (Plate XXII, fig. 1.)

*Beans.* Beans also ceased growth at the growing points after seven or eight weeks, the young undeveloped leaves becoming pale coloured and flecked with brownish intervenal spots, but the lower part of the plants remaining healthy for many weeks. (Plate XXII, fig. 2.)

*Clovers.* Clovers grew well for about eight weeks, and had formed short horizontal stems before symptoms of manganese deficiency were observed. It was then noted that the tips of the stems ceased growth, the young leaves became somewhat yellowish and developed brown flecks upon them, and later died from the margins inwards. The further development of the plants was checked owing to the collapse of the growing points of the stems and the apparent inability to develop further stems. Finally the plants in the manganese-free jars died completely. (Plate XXII, fig. 3.)

*Oats.* With Algerian oats the first symptoms of manganese deficiency in water-cultures in the glass-house appeared about four weeks (sometimes less) from the date of germination. The symptoms were a discoloration and collapse of the tissues of the leaf-blade in a characteristic manner. The trouble was usually first visible in a thin strip at each edge of the blade at a position about an inch from the leaf-base, and soon extended across the blade so that the tip three-quarters of the leaf fell over with a sharp kink at the collapsed portion. The first formed leaf was never affected in this manner, but the second, third and later leaves became affected successively, the distal end of the leaves remaining green for a considerable time after the lower portion had collapsed. (Plate XXIV, fig. 1.) On older leaves the collapse was not so markedly confined to the lower quarter and oval spots of collapsed tissue appeared irregularly on the leaf-blade, though less frequently towards the tip end. Streaks of tissue collapsing at the margins of the leaves were also very characteristic. (Plate XXIV, figs. 2 and 3.)

Finally new leaves ceased to push up through the leaf-base of the uppermost leaf, indicating death of the growing point, and all leaves shrivelled gradually and the plant completely died. (Plate XXIII, fig. 2.) It was pointed out in a previous communication (1928) how these

## 502 *Manganese as an Essential Element for Plant Growth*

symptoms of manganese deficiency in water-cultures corresponded exactly with the symptoms of Grey Speck disease of oats in the field.

The symptoms described above were those observed when oats were grown in a culture solution completely free from manganese. When manganese sulphate was added to make the concentration of manganese one part in fifty million, the amount proved to be inadequate for the growth of six seedlings to maturity without change of solution, but healthy growth and abundant tillering occurred for the first nine weeks. After this the characteristic marginal strips and spots of collapsed tissue, situated mainly on the basal half of the younger leaves, again indicated manganese deficiency. No symptoms of chlorosis, in the sense of yellowing of leaves, were at any time observed in connection with manganese deficiency of oats.

*Wheat and barley* did not show such characteristic symptoms as oats. The first symptoms were not noticed until a somewhat later stage, when three or four short tillers had been formed and the plants were five or six inches high. Streaks of collapsed tissue appeared on the younger leaves, running especially between the veins, and again situated mainly on the basal half of the leaf. In some cases these intervenal streaks were whitish, due to collapse of the mesophyll tissue. These whitish intervenal streaks were much more pronounced on the leaves of *maize* plants in manganese-free solutions, and closely correspond with those figured by Lee and McHargue (1928) for Pahala blight, or manganese deficiency, of sugar cane. Few new leaves were formed in any of these cereals after the appearance of deficiency symptoms, those which did grow out soon succumbing in a similar manner, and the plants remained dwarf for some weeks and ultimately gradually died.

*Grasses.* Grasses in most cases died at such an early stage in manganese-free solutions that the detailed record of symptoms was difficult. (Plate XXII, fig. 4, and Plate XXIII, fig. 4.) However, in the seedlings which attained a small size similar symptoms were noted to those described for the cereals. Irregular patches of collapsed tissue, frequently marginal, or intervenal streaks of collapsed tissue, appeared along the leaf-blades, followed by cessation of growth and later death.

*Tomatoes.* Tomatoes behaved somewhat differently in that the growing points of the plants in manganese-free solutions did not definitely die as in other cases, but the plants remained very dwarf, spindly, and almost stationary at about four inches high. The leaves formed showed a characteristic intervenal chlorosis as described and figured by Schreiner and Dawson (1927) and by Miller (1928).

It will be seen from the above that the general symptoms of manganese deficiency are a sudden cessation of growth with collapse of portions of the immature tissues, and gradual death of the growing point while the lower part of the plant remains alive, green, and apparently healthy for weeks. In dicotyledons a poor development of chlorophyll in the immature tissues gives a chlorotic appearance to the growing point. Leaves not quite fully developed frequently acquire a characteristic appearance owing to the intervenal mesophyll becoming yellow and leaving the veins standing out as a network of green.

In cereals and grasses the leaf symptoms are the more evident, owing to the growing point of the stem not being visible. Long whitish intervenal streaks in which the chlorophyll is not developed as in maize, or definite irregular patches of collapsed mesophyll tissue as in oats, are the two main types of symptoms to be observed.

It is desired to emphasise the fact that these symptoms are much more than a chlorosis in the usual sense of the word, and they cannot possibly be confused with chlorosis due to lack of iron (see the section on *Symptoms* in the Introduction). In other words, the effect of manganese deficiency is essentially an effect on the immature tissues at the growing point, although non-development of chlorophyll in these tissues may also be a characteristic.

The anatomical changes of which these symptoms are the expression will be described in detail in a further communication.

## B. EXPERIMENTS WITH OATS.

Oats were chosen for a more extended series of water-culture and pot experiments on account of the characteristic symptoms of manganese deficiency which they exhibit. This is a very great advantage, since with the majority of deficiency diseases the symptoms cannot be considered so specific that the deficiency can be named at a glance. With oats, moreover, the symptoms appear early—in four weeks or less from the date of germination if the nutrient solution is free from manganese. And also the manganese requirement of oats appears to be fairly high in comparison with that of some other plants, as will appear from the following experiments.

### (a) *The non-replaceability of manganese with other elements.*

To each of ten jars containing the manganese-free nutrient solution a different element was added as shown below, and control jars with and without manganese completed the series.

## 504 *Manganese as an Essential Element for Plant Growth*

(1) + B	1 : 2,500,000	as $H_2BO_4$	(7) + Ba	1 : 2,500,000	as $BaCl_2$
(2) + Al	"	" $Al_2(SO_4)_3$	(8) + Sr	"	" $Sr(NO_3)_2$
(3) + Zn	"	" $ZnSO_4$	(9) + I	"	" $KI$
(4) + Cu	"	" $CuSO_4$	(10) + Si	"	" $K_2SiO_3$
(5) + Co	"	" $Co(NO_3)_2$	(11) + Mn	"	" $MnSO_4$
(6) + Ni	"	" $Ni(NO_3)_2$	(12)	Nutrient solution alone	

Six plants were grown per jar. Within from six to seven weeks the plants in all jars except that containing manganese were showing the typical dying of the leaves characteristic of manganese deficiency as exhibited in jar 12. The series was discarded after twelve weeks' growth, when the plants in all jars were rapidly dying back from manganese deficiency except those in jar 11 containing manganese sulphate, which were vigorous and healthy.

In another series a combination of the elements zinc, copper, boron and aluminium (each in dilution 1 : 10,000,000) was used. Four jars were prepared with these elements added to the manganese-free culture solution, and to two of them manganese 1 : 5,000,000 was added in addition. The seedlings in the two jars with Zn, Cu, B, and Al, but without manganese, developed symptoms of manganese deficiency in about four weeks, whereas those in the jars containing manganese grew perfectly normally.

### (b) *The effect of different concentrations of manganese in the nutrient solution.*

Several investigators (Aso, Brenchley (1927) *et al.*) have determined the upper limits of concentration of manganese for barley and peas in water-culture. In dealing with the lower limits they have only reported a "stimulant" action observable at high dilutions. If manganese is an essential element as proved above, this stimulative effect is probably only due to a sufficiency of the element having been supplied for maximum growth under the existing conditions, there having been an insufficiency in the normal culture solution.

To investigate the lower limits with a culture solution known to be absolutely manganese-free, the following series with oats was therefore arranged (in triplicate):

Mn-free,  
Mn 1 : 50,000,000,  
Mn 1 : 10,000,000,  
Mn 1 : 5,000,000,  
Mn 1 : 1,000,000.

The plants in the manganese-free jars were showing symptoms of manganese deficiency within four weeks, and were never able to grow more than a few inches high. Those in the jars with manganese 1 : 50,000,000 grew well and tillered as freely as any of the plants in solutions of higher manganese content until about eight weeks old, when they suddenly developed symptoms of manganese deficiency. None of the other plants showed evidence of lack of manganese at the end of ten weeks' growth, at which time the solutions were changed to supply sufficient of the general nutrient salts for continued growth. The change of solution, with its fresh supply of manganese, permitted the plants in the 1 : 50,000,000 jars to shoot ahead again, and fresh healthy leaves appeared which developed no further deficiency symptoms until some four weeks later. Soon after this, however, the second change of solution again permitted further healthy growth, and at the time of harvesting these plants were nearly three feet high and producing a few ears. No symptoms of manganese deficiency were at any time observed on the plants in solutions with 1 : 10,000,000 of manganese or more. The dry-weights of the whole series when harvested were 14, 118, 241, 270 and 257 gm. respectively for the various concentrations. These figures would indicate that concentrations of one part of manganese in from one to five million parts of solution are the optimum for the growth of oats in water-cultures in which the solutions are changed only once or twice during the growing period. It would appear probable, however, that if the solutions were changed more frequently one part of manganese in ten million parts of solution would give as good a growth, and that if a method of continuous solution change was arranged, oats might be grown to maturity in a culture solution containing as little as one part of manganese in fifty million parts or more of solution.

(c) *The effect of removing manganese at different stages of growth.*

Twelve jars of oat seedlings were started in the nutrient solution with addition of manganese 1 : 500,000. After four weeks the plants in three jars were removed, their roots washed with distilled water, and the plants replaced in a manganese-free culture solution. Three more jars were similarly removed to a manganese-free solution after six weeks, and three more after eight weeks, the remaining three being grown on continuously in the manganese-containing solution, with one change of solution after ten weeks' growth.

The plants removed to manganese-free solution after four weeks' growth showed no evidence of manganese deficiency until they were ten

## 506 *Manganese as an Essential Element for Plant Growth*

to eleven weeks old. These plants managed to grow on, showing symptoms of manganese deficiency on all the upper leaves, and finally formed some heads at about 2 ft. 6 in. high. The plants had fewer tillers and were 6-9 in. shorter than normal plants.

It is probable that the amount of manganese these plants were able to absorb during their first four weeks' growth was just insufficient to enable them to reach maturity in a normal healthy manner. This point is further discussed in section C (c), dealing with the minimum quantity of manganese necessary for complete development.

The plants removed to manganese-free solutions after six and eight weeks' growth, and those grown continuously in the solution containing manganese, at no time showed evidence of deficiency and grew to a height of over three feet and flowered well.

### (d) *Comparison of oats and rye as to manganese requirement.*

The fact that the strip of rye sown along the roadside by the farmers of Mount Gambier develops to maturity where oats would fail, makes it interesting to determine whether this is due to a lesser manganese requirement on the part of rye, or whether it is due to a greater absorption of manganese by this crop under the existing conditions.

The former possibility was capable of test by water-cultures. It was known from a previous experiment that the amount of manganese supplied in a water-culture in which the concentration of this element was 1 : 50,000,000 was insufficient for the normal development of six oat seedlings. Accordingly two jars each of Algerian oats and rye were set up, each containing the standard nutrient solution plus manganese 1 : 50,000,000, with four seedlings per jar.

After several weeks' growth the oats developed pronounced symptoms of manganese deficiency, the leaves dying in the typical manner, no young leaves appearing, and the plants never attaining a height of more than one foot. They were not able to produce flowering heads. The rye, on the other hand, progressed well, showed no symptoms of manganese deficiency, and came into flower at a height of over two feet.

Further work is now being undertaken to determine with more precision the relative requirements for manganese of certain plants. The above experiment gives very suggestive indications, as does also the table of chemical determinations of manganese in plants from manganese-deficient soils (Table VI).

C. CHEMICAL DETERMINATIONS OF THE MANGANESE CONTENT OF OATS  
GROWN UNDER DIFFERENT CONDITIONS.

The above series of water-culture experiments raise interesting questions as to the manganese content of oat plants, its variability, and the possibility of there being an amount of manganese which must be regarded as the minimum quantity essential to support healthy growth to maturity. The following three sections provide information gained upon these points by systematic chemical work on oat plants from the field and from the water-cultures. The method used for the chemical determination of manganese is given in Appendix II.

(a) *Variation in manganese content with growth (oats and barley).*

In order to follow the changes in manganese content of oat plants during their period of growth under natural conditions, duplicate samples were taken at intervals of ten days throughout the growing period from a plot of Algerian oats in the Waite Institute Experimental Field. The plot was about 100 yards long, and each sample was obtained by cutting several plants at intervals of about ten yards along the length of the plot, and bulking these for analysis. The duplicate samples, taken adjacent to one another at each sampling, were always in good agreement.

The plot was sown on June 8th, 1928, 65 lb. of seed and 92 lb. of superphosphate being used per acre. The oats made slow growth at the start and the first sample was not collected until August 8th. The flowering stage was reached on October 17th and the plot was harvested on November 29th. The grain and straw samples were collected during harvesting.

The plot yielded at the rate of 4 tons 2 cwt. total produce, and 81.2 bushels of grain per acre.

Table II shows the percentage of crude ash and the amount of manganese expressed as parts per million parts of dry matter. The figures given are the average of the duplicate samples except that for October 17th which is the average value of 32 individual plants taken on this day.

The most noticeable feature of the manganese determinations is the increase in the proportion of this element between about the twelfth and sixteenth weeks of growth. From then there was a steady decline until the plant was ripe, but even then the proportion of manganese is only a little less than at the time of the first sampling.

## 508 *Manganese as an Essential Element for Plant Growth*

Table II.

*Showing the ash and manganese content of oats at different stages of growth.*

Date sampled (1928)		% Ash on dry matter	Mn p.p.m.
(Sown)	June 8th	—	—
	Aug. 8th	11.59	71.2
	18th	11.30	58.5
	28th	10.25	70.5
	Sept. 7th	10.83	68.8
	17th	10.46	80.1
	27th	10.38	92.4
	Oct. 8th	8.95	91.7
	(Flowering) 17th	7.56	81.1
	27th	7.38	82.7
	Nov. 6th	6.91	76.6
(Harvested)	29th (whole plant)	6.17	63.8
	29th (grain)	4.03	62.7
	29th (straw)	7.32	65.7

It remains to be determined whether these figures will show much seasonal variation. The average manganese content of three samples of Algerian oats taken from different plots at the Waite Institute during 1927 was 67.3 p.p.m., the samples being collected ten to twelve weeks after seeding. This agrees with the amount found at the same stage during 1928. But the manganese content of oat grain has varied from 40.3 p.p.m. in 1927 and 50.9 p.p.m. in 1926 to 62.7 p.p.m. in 1928. Similarly the manganese content of ripe plants of Algerian oats was only 22.4 p.p.m. in 1927 as against 63.8 p.p.m. in 1928.

Some samples of barley grown in pots during 1926 and harvested at different stages of growth were available and the manganese was determined in these. The amount found is shown in Table III.

Table III.

*Showing the ash and manganese content of barley at different stages of growth.*

Date sampled (1926)	% Ash on dry matter	Mn p.p.m.
Sown: May 12th	—	—
July 19th	17.70	57.3
Aug. 9th	17.68	41.7
Aug. 30th	15.00	40.7
Sept. 20th	12.17	33.9
Oct. 11th	9.23	26.1
Nov. 9th	8.88	26.4



It is seen that in this case there was a continuous decrease in the proportion of manganese to dry matter as the plants approached maturity. Whether such a marked decrease would also have been found in plants taken from the field is as yet uncertain.

(b) *The variability in manganese content of individual oat plants.*

The plot of Algerian oats mentioned in the preceding section was sampled at the flowering stage, by cutting 25 plants spaced at approximately equal intervals along its length. The individual plants were kept separate and analysed for ash and manganese to determine the variation

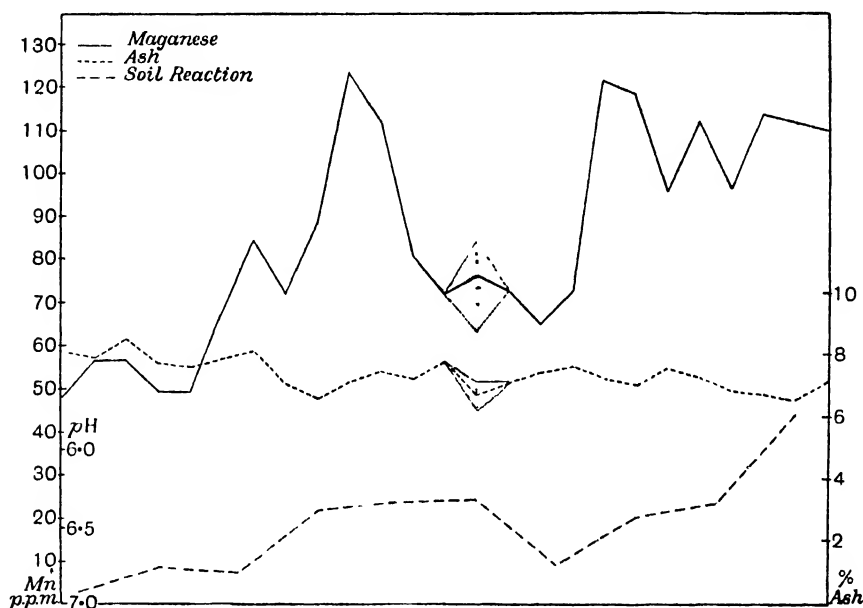


Fig. 2. Graph showing the variation in manganese and ash in 25 individual oat plants (variety Algerian) taken at approximately equal intervals along a field plot. In the centre the variation for eight plants taken from a small area about eight inches square is shown. The variation in soil reaction along the plot (taken some months later) is also indicated.

from plant to plant throughout the plot. Near the centre eight plants were taken from a small area about eight inches square to determine the natural variation in plants growing at the same spot. The manganese content of these eight plants growing alongside each other varied from 63.0 to 84.2 parts of manganese per million parts of dry matter and

## 510 *Manganese as an Essential Element for Plant Growth*

averaged 76.2 p.p.m. From the separate values the probable error of any single determination was calculated and found to be 6 per cent. This may be taken as the probable error of the other single plant samples.

The results of all the determinations are shown diagrammatically in Fig. 2, the amounts of manganese and ash in the individual plants being plotted in relation to the approximate position of the plant in the plot.

It will be seen that there is a progressive variation in manganese content throughout the plot. The first few samples are relatively low in manganese. There is then an increase reaching a maximum in the tenth sample. Near the centre of the plot the amount of manganese is definitely lower but not as low as in the first five samples. The last eight plants are again much richer in manganese.

The large variation in the manganese content throughout the whole plot (48.3–123.7 p.p.m.) is believed to be mainly due to two factors, namely the soil reaction and the flooding of the plot by some of the winter rains. Unfortunately soil samples to determine the reaction were not taken until some months after the plant samples, and so it was not possible to take them from the exact spot on which the plant was growing. Ten samples were taken at approximately equal distances along the plot and the *pH* determined by the quinhydrone electrode, the values found being shown in the diagram (Fig. 2).

It will be seen that considerable variation in soil reaction occurs from point to point along the plot, the maximum difference found being 1.1 *pH* units. There is some correlation between the curves of the manganese content of the plant and the soil reaction, the higher manganese figures being associated with the more acid soil conditions.

However, the uneven flooding of the plot by some of the winter rains and the resultant water-logging of the soil have had an effect which it is difficult to evaluate. Further investigations, in which the soil and plant samples are taken at the same time and from the same place, are needed to establish the correlation between soil reaction and the manganese content of the plant.

Owing to the variation in soil reaction found in the apparently uniform plot investigated, it would appear that in taking plant samples for manganese determinations from ten to twenty plants should be cut from an area of a few square yards, similar samples being taken from at least two other places in the field. A sample of soil should also be taken from each place and the reaction determined.

(c) *The minimum amount of manganese found in healthy oat plants at the flowering stage.*

From the great variation in the manganese content of healthy oats grown at different places, it appears that in a large number of cases there must be far more manganese absorbed than is actually needed for normal growth. It was thought that the analysis of the tops of oats grown in the water-cultures with the smaller concentrations of manganese would give an indication of the minimum amount of that element to be expected in healthy plants. Table IV shows the manganese content of some of the oats grown in water-cultures during 1927 and 1928. At the time of harvesting, the 1928 series was at the flowering stage and the 1927 series was somewhat more advanced. In each case the oats grown in the manganese-free nutrient solutions had died at much earlier stages.

Table IV.

*Showing the manganese content of Algerian oats from water-cultures.*

Year	No.	Mn in nutrient solution	Mn in plants p.p.m. dry matter	Mean p.p.m.	Severity of Mn deficiency symptoms
1927	A 1-4 and C 2-4	Nil	—	6.2	Died as seedlings
1928	B 1-3	Nil	—	12.3	„
	B 4 } 5 } 6 }	1 : 50,000,000	{ 7.7 } { 8.9 } { 9.0 }	8.5	Badly diseased
	F 1 } 2 } 3 }	*	{ 12.3 } { 11.3 } { 12.7 }	12.1	Diseased
	B 7 } 8 } 9 }	1 : 10,000,000	{ 14.05 } { 13.9 } { 16.35 }	14.8	Healthy
	B 10 } 11 } 12 }	1 : 5,000,000	{ 21.4 } { 19.0 } { 23.5 }	21.3	„
1927	C 5 } 6 } 7 } 8 }	1 : 4,000,000	{ 18.3 } { 23.9 } { 18.0 } { 23.6 }	21.0	„
1928	B 13 } 14 } 15 }	1 : 1,000,000	{ 73.4 } { 82.5 } { 92.9 }	82.9	„
1927	C 9 } 10 } 11 } 12 }	1 : 400,000	{ 111 } { 103 } { 85.2 } { 83.7 }	95.7	„

\* Plants in Mn 1 : 500,000 for 1 month and then transferred to Mn-free nutrient solutions.

## 512 *Manganese as an Essential Element for Plant Growth*

From the foregoing results it is seen that the oats which died at an early stage of growth from lack of manganese contained 6.2–12.3 parts of manganese per million parts of dry matter. Of the plants which exhibited characteristic symptoms of the disease but were still alive at harvesting (B 4–6 and F 1–3) none contained more than 12.7 p.p.m. of manganese.

The lowest manganese content of healthy oats was found in those plants growing in jars B 7–9 and averaged 14.8 p.p.m. The total growth in these jars, although quite healthy, was not as great as in jars B 10–12, and it would thus appear that the dry weight of the crop produced was limited by the amount of manganese supplied in the nutrient solution. Therefore it seems likely that the proportion of manganese found in the dry matter of these plants is approximately the minimum quantity to be expected in healthy oats at this stage of development.

When the amount of manganese in the nutrient solution was 1 : 4,000,000 (1927) and 1 : 5,000,000 (1928) the amount of manganese in the dry matter averaged 21 p.p.m. In each season optimum growth was obtained in these jars. With nutrient solutions of greater manganese concentration larger amounts of manganese up to 83–96 p.p.m. were found in the dry matter, but in each case the growth was practically the same as at the lower concentration. Thus at least five to seven times as much manganese as appears to be necessary for growth can be present without affecting the plant in either way.

A number of samples of Algerian oats from different localities were examined to determine how their manganese content compared with that of the plants grown in water-cultures. Table V shows the localities selected and the amount of manganese found.

Table V.

*Showing manganese content of Algerian oats at flowering stage.*

Locality			Mn p.p.m.	Remarks
(1)	Penola	...	10.2	Diseased
(2)	Mt Gambier	...	10.4	"
(3)	Mil Lel	...	10.3	"
(4)	Mil Lel	...	14.3	Diseased but recovered somewhat
(5)	Wasleys	...	20.4	Healthy
(6)	Bundaleer Valley	...	24.7	"
(7)	Riverton	...	35.7	"
(8)	Belalie North	...	47.5	"
(9)	Waite Institute	...	82.0	"

The first three samples represent oats, all of which showed marked symptoms of manganese deficiency. Sample 4 was from a self-sown crop of Algerian oats across the road from sample 3. In its earlier stages this self-sown crop had exhibited symptoms of the disease, but with the approach of spring had recovered somewhat and produced some grain. Samples 5-9 represent oats from localities not subject to the manganese deficiency disease. From the analyses it will be seen that there is the same range of variation in manganese content in the last five samples as in the samples of healthy oats from the water-cultures.

The amount of manganese in sample 4 was almost identical with the minimum amount found in the water-culture plants that were making healthy growth, and is further evidence that this amount (about 14 p.p.m.) is the minimum quantity to be expected in normal Algerian oats at the flowering stage.

#### D. EXPERIMENTAL WORK RELATING TO FACTORS POSSIBLY INFLUENCING THE APPEARANCE OF MANGANESE DEFICIENCY IN OATS IN THE FIELD.

The following series of water-cultures was designed to test whether excess of calcium ions or the presence of traces of organic substances, as were claimed by Arrhenius (1924) and by Hudig (1923) respectively to influence the appearance of Grey Speck disease of oats in the field (which was shown by us (1928) to be identical with manganese deficiency), would also have an influence on the availability of manganese in water-cultures.

##### (a) *The effect of excess of calcium ions in water-culture.*

Solutions with excess of calcium ions in different proportions were prepared in the following way:

The sodium chloride of the standard solution was reduced to 0.1 gm. per litre, and calcium chloride was added to raise the proportion of calcium ions to the four ions Ca, Mg, Na, K, to the desired degree. In one series the proportion of calcium ions was raised to 80 per cent., the solution being used diluted to quarter strength. In another series the proportion of calcium ions was raised to 50 per cent., the solution being used both undiluted and diluted to two-thirds strength. Three similar series were prepared in which calcium nitrate was added instead of calcium chloride.

Three jars of each solution were prepared, to two of which manganese 1 : 5,000,000 was added, the third being left manganese-free.

## 514 *Manganese as an Essential Element for Plant Growth*

All plants in the manganese-free jars showed symptoms of manganese deficiency within four to six weeks as usual, but at no time did symptoms of manganese deficiency appear on any of the plants in jars containing manganese, however high the proportion of calcium ions (Plate XXIII, fig. 2). Excess of calcium ions therefore does not render manganese unavailable if this is present in the soluble and highly ionized form of sulphate (or probably of any soluble inorganic manganese salt).

### (b) *The effect of excess of nitrate ions in water-culture.*

It has been noted in many field experiments on the Grey Speck disease of oats that nitrate of soda tends to increase the severity of the disease. Nitrate of soda was added to six jars of manganese-free culture solution (in which the sodium chloride was reduced to 0.2 gm. per litre) to the extent of 0.15 per cent. in three jars and 0.3 per cent. in three jars. To two jars of each series Mn 1 : 5,000,000 was added.

Again the plants in the manganese-free jars developed typical symptoms of manganese deficiency, whereas all plants in jars containing manganese grew normally, notwithstanding the presence of a considerable quantity of nitrate of soda.

### (c) *The effect of the presence of organic compounds.*

The following water-culture series was arranged:

G 1-3	+ "humus" from Mn-deficient soil	2 Mn 1 : 5,000,000 jars, 1 Mn-free jar
G 4-6	+ "humus" from sugar	" "
G 7-9	+ sucrose, 0.05 %	" "
G 10-12	+ dextrose, 0.05 %	" "
G 13-15	+ starch, 0.05 %	" "
G 16-18	+ cellulose, 0.05 %	" "
G 19-21	free of organic matter	" "

As was to be expected, there was a growth of fungi over the surface of the solutions containing sugars, but this did not interfere with the determination of symptoms of manganese deficiency. Typical symptoms of manganese deficiency appeared in all the plants grown in manganese-free solutions, but were not at any time observed on plants in solutions containing manganese sulphate, whichever of the above organic substances was present in the nutrient solution.

It is thus evident that the factors, (1) excess of calcium ions, (2) excess of nitrate ions, or (3) the presence of traces of organic compounds in the nutrient solution, are not in themselves the cause of manganese deficiency in oats. It remains possible, however, that they may act in combination

with some other factor or factors as yet undetermined in rendering manganese unavailable in certain soils.

\*(d) *Chemical determinations of manganese in plants from manganese deficient soils as compared with that in plants from normal soils.*

To strengthen the argument advanced in our previous paper (1928), derived from soil percolation tests, that manganese is less available in the soils on which oats suffer from manganese deficiency, the following series of analyses was done on various plants and weeds, all of which, with the exception of oats and barley, appeared just as healthy and vigorous on the manganese deficient soil as on the normal soil.

The normal soil from which the plants were collected was the red clay loam at the Waite Institute, on which no deficiency disease appears. Corresponding samples of plants were always selected at the same stage of growth.

Table VI.

*Showing the manganese content of plants from normal and manganese deficient soils.*

Results expressed as parts of manganese per million parts of dry matter.

Plant	Mn content when growing on a normal soil. (Waite Institute) p.p.m.	Mn content when growing on Mn- deficient soil p.p.m.	Locality from which Mn-deficient sample obtained
Algerian oats, 11-15 weeks after seeding	73.1*	{ 7.1† 18.5‡	Penola Mt Gambier
Algerian oats, nearly ripe ... ..	76.6	{ 10.2 13.0§	Penola Mt Gambier
Lachlan oats, flowering ... ..	71.4	13.2§	Mt Gambier
<i>Cryptostemma</i> , <i>Calendulaceum</i> (Capeweed)	72.1	30.9	"
<i>Poa pratensis</i> ... ..	37.0	30.3	"
Barley ... ..	26.4	7.65†	Corney Point
<i>Bromus maximus</i> ... ..	57.1	11.6	"
<i>Sonchus oleraceus</i> ... ..	74.1	16.8	"
Lucerne ... ..	—	17.4	"
<i>Lolium temulentum</i> ... ..	—	7.5	"
Perennial rye grass ... ..	—	11.5	Mt Gambier

\* Average of 6 samples.

† Average of 4 samples (diseased).

‡ Average of 5 samples (diseased).

§ Average of 2 samples (diseased).

|| Grown in pots (1926).

From the table it will be seen that the manganese content of all the plants examined from the Waite Institute was much greater than that of the same species growing at any of the other three places. The difference was least in the samples of *Poa pratensis*, the Waite Institute

sample being only 21 per cent. richer in manganese than the sample from Mt Gambier. The greatest difference was shown in the oats from Penola and the Waite Institute, the latter samples containing on the average ten times as much manganese as the former. In all other cases the samples taken from the Waite Institute contained  $2\frac{1}{2}$  to  $7\frac{1}{2}$  times as much manganese as the corresponding sample from a manganese deficient soil.

These differences are all of a greater order than the normal variation from plant to plant as found in the examination of single oat plants, and, as composite samples were always taken so as to represent the average for the particular locality, the variations found must be taken to represent variations in the availability of manganese in the different soils.

### III. DISCUSSION.

The two most important points brought out by the above work are: (1) that manganese is an essential element for the growth of all the plants tested, being essential from an early seedling stage, and (2) that different plants require different amounts of manganese to enable them to complete their development. The latter is the explanation of the fact that certain types of soil, which do not possess sufficient available manganese for the growth of oats, nevertheless support an apparently normal growth of pasture plants and weeds. At the same time such pasture plants and weeds have a considerably lower manganese content than similar plants from normal soils. It seems possible that this fact may later be found to have some connection with certain animal diseases which occur on these manganese deficient soils in South Australia.

The question of what factor or factors is responsible for the poor availability of manganese in these soils is still unsolved. Oats have been found to suffer from manganese deficiency on three widely differing types of soil in South Australia. These are: (1) a rich, brown, volcanic ash soil from round Mounts Gambier and Schank; (2) a black clay-humus reclaimed swamp soil from Penola; and (3) a light calcareous soil from the foot of Yorke's Peninsula, Eyre's Peninsula and Kangaroo Island.

That the poor availability of manganese on these soils is connected with the soil reaction becomes clear from the fact that the patches on which oats are most badly diseased are always more alkaline in reaction, and from the fact that liming increases the severity of the disease. There are many other soils as alkaline as these, however, and possessing no more manganese, on which the trouble does not appear.

Moreover, under certain conditions the soils in which manganese is normally unavailable in sufficient quantity for the growth of oats, may



so change, without manurial treatment, that abundant manganese becomes available.

An illustration of this is furnished by a series of pot experiments which was designed to investigate the effect of soil reaction on the manganese deficiency disease of oats. The series consisted of sixteen (glazed) pots of soil from a field at Mt Gambier on which oats suffered from manganese deficiency. The soil for these pots was treated with hydrochloric acid or calcium carbonate in such a way as to produce a range of hydrogen ion concentration from  $pH$  5.5 to  $pH$  8.0 in eight steps, there being two pots of each reaction.

Table VII.

*Showing an unexpected result in a series on the influence of soil reaction on manganese deficiency disease of oats.*

Pot no.	Treatment	$pH$ expected	$pH$ found (14. vi. 28)	Remarks	Mn in crop (3. x. 28) p.p.m.
1 and 2	187.6 ml. HCl	5.5	5.6	Healthy	290
3 and 4	141.5 "	5.9	6.0	"	90.4
5 and 6	92.3 "	6.4	6.9	"	14.4
7 and 8	67.7 "	6.7	7.0	"	8.8
9 and 10	39.0 "	7.1	7.4	Diseased }	11.4
11 and 12	20.5 "	7.4	7.5		
13 and 14	Untreated	7.7	8.0	Healthy	115.8
15 and 16	30.75 g. $CaCO_3$	8.0	8.2	"	134.5

From experience, as detailed in our previous paper (1928), it was expected that the manganese deficiency disease would appear on the oats in all pots with a reaction more alkaline than about  $pH$  7.0. It was expected that the trouble would be more severe in the pots treated with calcium carbonate than in the untreated pots; that it would be slightly less severe in the pots treated with such small amounts of acid as to leave the reaction still on the alkaline side; and that it would not appear at all in the pots made more acid than about  $pH$  7.0.

As was anticipated (see Table VII) the disease did not appear in the pots more acid than  $pH$  7.0; it did appear in the pots of reaction  $pH$  7.4–7.5, which had been treated with small amounts of acid, but it did not appear, where it had been expected that it would, in the untreated pots ( $pH$  8.0), or in those treated with calcium carbonate ( $pH$  8.2). The plants were harvested after fifteen weeks' growth and the manganese present in the tops determined. Corresponding with the deficiency symptoms, it was found that the plants from the slightly acid-treated

pots which showed the disease had little manganese, whereas the plants from the untreated and the calcium carbonate treated pots, which had remained healthy, had much more manganese.

This unexpected result can only be explained by a difference in the method of filling the untreated and calcium carbonate treated pots from the acid treated ones. The soil for the whole series had been air-dried and sieved, and the various amounts of acid required, diluted in a definite volume of water, had been thoroughly mixed into the soil by hand stirring so that an evenly moist sample was obtained before filling into the pots. The measured amount of calcium carbonate was thoroughly mixed with the air-dry soil in a revolving box, and this, and also the soil for the untreated pots, was filled into the pots dry. When these pots were watered the soil must have become packed very tightly as it swelled, and this appears to have greatly influenced the availability of the manganese.

In this connection the experience of Godden and Grimmett (1928) that the manganese content of oats in undrained pots was about six times that of oats in drained pots is interesting.

It has also been noted in field experiments on manganese deficient soils in South Australia that rolling is beneficial for the growth of oats, and if the crop has been sown with the aid of a tractor the improved growth in the track of the tractor wheels is most noticeable.

These examples would suggest that the oxidation reduction potential of the soil is well worth investigating in further work on the factors influencing availability of manganese in soils.

A further point worthy of attention arises from a consideration of the *symptoms* of manganese deficiency. The arrest of shoot growth, with formation of small chlorotic leaves at the tips, and slightly lower down the characteristic green network of veins with yellowed mesophyll tissue between, recall at once the symptoms of several well-known diseases the cause of which has long been obscure. Pecan rosette, mottle-leaf of citrus, and little-leaf or yellows of walnut are perhaps the three most important diseases thus called to mind. No tests were done with these plants in the present investigation, and so no more detailed discussion of the symptoms can be undertaken here. But besides the apparent similarity between the symptoms of these diseases and those of certain other plants known to be suffering from manganese deficiency, there is also some similarity in the mode of occurrence. There is evidence that both pecan rosette and mottle-leaf of citrus are made worse by applica-

tions of lime, and that walnut yellows is increased on plots heavily treated with nitrate of soda for some years. Certain relations to the amount of humus present in the soil also correspond with what has been found for some naturally occurring manganese deficiency diseases. At all events, the similarities which exist suggest that it would be fully worth while to determine by experiment whether the pecan rosette, the mottle-leaf of citrus and walnut yellows are not possibly manganese deficiency diseases.

The writers desire to acknowledge their indebtedness to Prof. J. A. Prescott for helpful criticism in the preparation of this manuscript.

#### IV. SUMMARY.

1. The conclusions of certain previous investigators that manganese is an essential element for plant growth have been confirmed by means of water-cultures.

2. Manganese becomes necessary to plants at an early seedling stage, and remains necessary until a late stage in growth.

3. The symptoms of manganese deficiency are essentially an arrest of development followed by death of the undeveloped tissues at the growing points, and the use of the word chlorosis as the primary symptom is misleading. In many cases a special type of chlorosis of the upper parts of the plant does occur before death of the tissues.

4. Rye developed to maturity in water-cultures on an amount of manganese which did not allow the complete development of oats.

5. A concentration of manganese of one part in fifty million parts of nutrient solution allowed complete development of rye, and would probably be sufficient for oats also if a method of continuous solution change was arranged.

6. Evidence is given that the minimum quantity of manganese which will allow healthy growth of Algerian oats is about 14 parts per million of the dry matter at the flowering stage.

7. On certain soils oats are unable to absorb this minimum quantity, and they then suffer from the Grey Speck disease, a true manganese deficiency disease which can be cured by application of a soluble manganese salt.

8. On soils with abundant available manganese considerably more manganese is absorbed by oats than is required for normal development. The amount absorbed varies greatly, and in all probability depends to some extent on soil reaction and soil aeration.

9. Analyses are given showing that a number of plants, when

## 520 *Manganese as an Essential Element for Plant Growth*

growing on a red clay loam soil on which no deficiency disease occurs, have a much higher manganese content than similar plants taken from soils on which oats suffer from manganese deficiency.

10. The variation in ash and manganese content during the growth of the oat plant is shown in Table II.

11. Such factors as the presence of organic matter, or excess of calcium ions, which have at various times been claimed as causes of the Grey Speck disease of oats have been shown to play no part in the appearance of this trouble in water-cultures where a soluble manganese salt is present. These factors cannot themselves render manganese unavailable when it is present in a soluble form. This does not exclude the possibility that they may later be found to have some effect, in conjunction with other factors as yet undetermined, in rendering manganese unavailable in certain soils.

12. None of ten different rarer elements tried, nor the combination zinc, copper, boron and aluminium, was able to replace manganese in the growth of oats.

### V. APPENDICES.

#### I. *Water-culture methods.*

The seed of cereals was germinated on waxed mosquito netting fitted over a jar of distilled water. The seed of smaller plants—clovers, grasses, tomato, etc.—was germinated in a pure sand which had been boiled with hydrochloric acid and well washed with distilled water. When the cotyledons had expanded the seedlings were washed out from the sand and transferred to the holes in waxed mosquito netting over a beaker of manganese-free culture solution until they were sufficiently big to set out in the culture jars.

The culture vessels used were rectangular museum jars of capacity 3.2 litres, covered on the outside with black paper and coated on the inside with a thin layer of paraffin wax. When solutions were changed fresh jars were used if the paraffin wax showed signs of flaking off. The rectangular shape of jar allowed plants to be readily lifted out for change to another jar, inspection of roots, etc.

The seedlings were supported by means of a little paraffined cotton wool in holes in rectangular wooden covers which had been treated with raw linseed oil and later dipped in melted paraffin wax. There were six holes per cover for seedlings, and one through which a bent aerating tube was fixed, this also being paraffined where it dipped into the solution.

Seedlings were set out in the culture jars at as small a stage as they could be conveniently handled. From one to six seedlings were used per jar, according to the experiment; in the majority of cases six was the number used.

The Rothamsted culture solution, with ferric citrate substituted for ferric chloride, was used throughout the work except for certain leguminous plants for which Brenchley's modified solution of reaction pH 6.2 was used.

*Precautions for the exclusion of manganese.* Ordinary distilled water from a Stokes

still was used in the making of all culture solutions. As described above, the insides of all culture jars, and the glass aerating tubes, were paraffined, so that solutions did not come in contact with glass after they were prepared. In an experiment in which oats were grown in a manganese-free culture solution in both paraffined and unparaffined jars, it was found that the plants in the unparaffined jars grew larger and lived longer than those in the paraffined jars, which were completely dead after some three months' growth. The controls with manganese 1 : 5,000,000 were equally vigorous in both, and were only discarded when three feet high. It seems probable that a still greater difference between plants grown in unparaffined and paraffined jars, due to absorption of manganese from the glass in the former, would have been evident if plants with a smaller manganese requirement than oats had been used.

Analytical reagents were used in the making of culture solutions, and these were all carefully tested and shown to be free from manganese by the periodate test. It was however found impossible to purchase an iron salt free from manganese. The presence of manganese in the purest reagent iron salts obtainable was shown both by the periodate test, and by water-cultures with plants. In an experiment in which oats were grown in a "manganese-free" culture solution prepared with iron salts guaranteed free of manganese as purchased, the plants grew much further than those in solutions really free from manganese, made with the specially prepared manganese-free iron citrate described below. Again it seems probable that if plants with a smaller manganese requirement than oats had been used the difference would have been considerably greater, and possibly no symptoms of manganese deficiency would have been evident at all with some plants in solutions prepared with ordinary A.R. iron salts.

*Preparation of manganese-free ferric citrate.* In order to obtain larger quantities of iron-free from manganese an electrolytic deposition method was used instead of the modified basic acetate separation noted in our previous paper (Samuel and Piper, 1928). The iron was deposited from a hot 14 per cent. solution of the purest obtainable ferrous sulphate on to a platinum cathode. The anode was a bar of iron separated from the cathode by a porous cell. This porous cell served to retain any manganese precipitated as the dioxide at the anode. The electrolyte was replaced every two or three hours by a fresh solution of ferrous sulphate. At the same time the iron, deposited on the cathode, was removed by solution in pure concentrated hydrochloric acid.

When sufficient ferrous chloride had been thus prepared it was recrystallised from the hot hydrochloric acid solution. It was then oxidised to ferric chloride by boiling with a small excess of pure nitric acid, this excess being removed by evaporation with a little hydrochloric acid on a water bath.

The ferric chloride so obtained was diluted to a definite volume and iron determined in an aliquot portion. In another portion, corresponding to 4–10 gm. of iron, the absence of manganese was proved using the periodate test.

The remainder of the ferric chloride solution was evaporated in a silica basin after the addition of sufficient pure citric acid to secure about two-thirds conversion to ferric citrate. This evaporation removed most of the free hydrochloric acid. The mixture of ferric chloride and citrate, left as a pasty mass, was dissolved in warm water, filtered, made to a definite volume, and kept in a paraffined flask. It was used in this form in the preparation of the nutrient solutions.

*II. Method for the chemical determination of manganese in plants.*

Composite samples have been taken by cutting plants as close to the ground as possible, precautions being observed to prevent contamination with soil. After air-drying the sample was hand-picked to eliminate dust and traces of soil. It was then oven-dried and ground in a Wiley Mill, using a 1 mm. screen. Manganese has been determined throughout by a colorimetric method after ashing and oxidation to permanganate. Owing to its superiority potassium periodate has been used to bring about this oxidation (Willard and Greathouse, 1917). A Dubosq type colorimeter with 100 mm. tubes has been used for making all colour comparisons. When insoluble matter, such as calcium sulphate, renders the colour solution turbid, it can easily be removed by centrifuging for 1-2 minutes immediately before making the colour comparison.

The details of the method are as follows:

5-25 gm., according to the amount of manganese present, of the oven-dried sample were ashed as completely as possible in a silica basin. The ashing was started over a small burner and finished in an electric muffle at a dull red heat.

The ash was treated with 25 ml. of dilute hydrochloric acid (1 + 1) and digested for 15 minutes under a clock glass on a water bath. It was then evaporated to dryness and left on the bath for about an hour to render silica insoluble. The residue was taken up with 25 ml. of hot water and 5 ml. of conc. nitric acid, filtered, and washed once or twice with hot water and two lots of warm dilute nitric acid. The washing was finished with hot water alone.

The filtrate was transferred to a silica basin and evaporated to dryness on a water bath. 25-30 ml. of dilute sulphuric acid (1 + 1) and 5 ml. of nitric acid were added and the evaporation continued on the water bath and finally on a hot plate or sand bath until fumes of sulphuric acid had been produced for about a minute.

When cold the contents of the basin were diluted with 30 ml. of water and 1-1.5 ml. of phosphoric acid. 0.3-0.5 gm. of potassium periodate was added and the solution in the basin boiled after the production of the permanganate colour. If the colour was sufficiently intense a further 25-30 ml. of water were added during boiling. The solution was then transferred to a suitable volumetric flask (50 ml., 60 ml. or 100 ml.) and diluted nearly to the mark. It was then placed in a boiling water bath for twenty minutes together with other flasks containing quantities of a standard manganous sulphate solution (from  $\text{KMnO}_4$ ), sulphuric and phosphoric acids, and the potassium periodate. Useful standard colour solutions ranged from 0.07 mg. to 1.0 mg. manganese per 50 ml.

When cold, and after diluting to the graduation mark, the colour of the permanganate is matched against one of the above solutions of known strength.

## REFERENCES.

- ARRHENIUS, O. (1923). Försök till bekämpande av havrens gräfläcksjuka. *Medd. Centralanst. försöksv. jordbr. Stockholm*, No. 244, 3-19.
- (1924). *Ibid.* Part II, Kärll och Faltförsök. *Ibid.* No. 256, 3-20.
- BISHOP, W. B. S. (1928). The distribution of manganese in plants and its importance in plant metabolism. *Austral. Journ. Exp. Biol. Med. Sci.* v, 125-42.
- BRENCHLEY, W. E. (1927). *Inorganic plant poisons and stimulants*. 2nd edition. Cambridge Univ. Press.
- GILBERT, B. E. and McLEAN, F. T. (1928). A "deficiency disease": the lack of available manganese in a lime induced chlorosis. *Soil Sci.* xxvi, 27-31.
- GODDEN, W. and GRIMMETT, R. E. R. (1928). Factors affecting the iron and manganese content of plants with special reference to herbage causing "pining" and "bush sickness." *J. Agric. Sci.* xviii, 363-8.
- HILTNER, E. (1924). Die Dörrfleckenkrankheit des Hafers und ihre Heilung durch Mangan. Das Kohlensäure-Mineralstoff-Gesetz, ein Beitrag zur Physiologie nichtparasitärer Krankheiten. *Landw. Jahrb.* lx, 689-769. Abs. in *Rev. Appl. Myc.* iv, 275-6.
- HUDIG, J. (1923). Diseases of crops on alkaline and sour soils. *Rpt. Int. Conf. Phytop. and Ec. Entom. Wageningen*, 137-8.
- LEE, H. A. and McHARGUE, J. S. (1928). The effect of a manganese deficiency on the sugar-cane plant and its relationship to Pahala blight of sugar-cane. *Phytopath.* xviii, 775-86.
- MAZÉ, P. (1914). Recherches de physiologie végétale. IV. Influences respectives des éléments de la solution minérale sur le développement du maïs. *Ann. Inst. Past.* xxviii, 21-68.
- McHARGUE, J. S. (1922). The rôle of manganese in plants. *J. Amer. Chem. Soc.* xliv, 1592-8.
- (1924). The association of manganese with vitamins. *J. Agric. Res.* xxvii, 417-24.
- (1926). Manganese and plant growth. *Ind. and Engl. Chem.* xviii, 172-5.
- McLEAN, F. T. and GILBERT, B. E. (1925). Manganese as a cure for a chlorosis of spinach. *Science*, lxi, 636-7.
- MILLER, L. P. (1928). Manganese deficiency in sand cultures. *Amer. Fertilizer*, March 31st, 1928.
- RIEHM, E. (1917). Nichtparasitäre Haferkrankheiten. Dörrfleckenkrankheit, Perchloratvergiftung. *Deutsche landw. Presse*, xliv, 62.
- SAMUEL, G. and PIPER, C. S. (1928). Grey Speck (Manganese deficiency) disease of oats. *J. Agric. South Australia*, xxxi, 696-705, 789-99.
- SCHREINER, O. and DAWSON, P. R. (1927). Manganese deficiency in soils and fertilizers. *Ind. and Eng. Chem.* xix, 400-4.
- SOMMER, A. L. and LIPMAN, C. B. (1926). Evidence on the indispensable nature of zinc and boron for higher green plants. *Plant Physiol.* i, 231-49.
- SOMMER, A. L. (1928). Further evidence of the essential nature of zinc for the growth of higher green plants. *Ibid.* iii, 217-21.
- WILLARD, H. H. and GREATHOUSE, L. H. (1917). Colorimetric determination of manganese by oxidation with periodate. *J. Am. Chem. Soc.* xxxix, 2366-77.
- WILLIS, L. G. (1928). Response of oats and soy beans to manganese on some Coastal Plain soils. *North Carolina A.E.S. Bul.* 257, 1-13.

## EXPLANATION OF PLATES XXII—XXIV

## PLATE XXII.

(The scale is given by the water-culture jars, which are 9" × 6".)

- |   |   |
|---|---|
| Fig. 1. Peas (Brunswick White), 10 weeks          | } On left, jar containing nutrient solution plus manganese 1 : 1,000,000 (as sulphate); on right, jar containing nutrient solution free from manganese. |
| Fig. 2. Broad beans, 15 weeks                     |   |
| Fig. 3. <i>Trifolium subterraneum</i> *, 16 weeks |   |
| Fig. 4. <i>Phalaris bulbosa</i> , 10 weeks        |   |

\* Manganese 1 : 1,000,000 was found to be toxic to the clovers, and after six weeks' growth the concentration of this element was reduced to 1 : 5,000,000, after which the plants grew forward in a healthy manner.

## PLATE XXIII.

(The scale is given by the water-culture jars, which are 9" × 6".)

- Fig. 1. Wheat (Late Gluyas) and barley (Cape), both 20 weeks old, in nutrient solution plus manganese 1 : 1,000,000, showing stage to which cereals were grown before harvesting.
- Fig. 2. Algerian oats, 18 weeks old, in solutions containing excess of calcium nitrate to raise the proportion of calcium ions to the ions Ca, Mg, Na, K to 50 per cent. On left, jar containing this nutrient solution plus manganese 1 : 5,000,000; on right, jar containing the nutrient solution free from manganese.
- Fig. 3. Barley (Cape), 16 weeks old. On left, jar containing nutrient solution plus manganese 1 : 1,000,000; on right, jar containing nutrient solution free from manganese.
- Fig. 4. *Bromus unioloides*, 10 weeks old. On left, jar containing nutrient solution plus manganese 1 : 1,000,000; on right, jar containing nutrient solution free from manganese.

## PLATE XXIV.

(Symptoms of manganese deficiency on oats.)

- Fig. 1. Oat seedling grown on a soil deficient in available manganese, showing the typical symptoms of manganese deficiency in the early stages. Note the first leaf healthy, second and third leaves collapsed below the centre of the blade.
- Fig. 2. Leaves from a plant of Algerian oats grown on a soil deficient in available manganese. Note the dead spots and streaks along the margins, characteristic of manganese deficiency on older plants. The presence or absence of a reddish margin to some of the spots is a variable feature, and is probably largely due to cold.
- Fig. 3. Leaves from plants of Algerian oats grown in water-cultures free from manganese. Note the dead spots and streaks along the margins and correspondence with field symptoms of manganese deficiency as in Fig. 2.

(Received May 10th, 1929.)





Fig. 1.



Fig. 2.

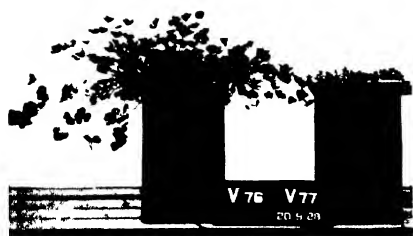


Fig. 3.



Fig. 4.

SAMUEL & PIPER.—MANGANESE AS AN ESSENTIAL ELEMENT FOR PLANT GROWTH (pp. 493-524.)





Fig. 1



Fig. 2

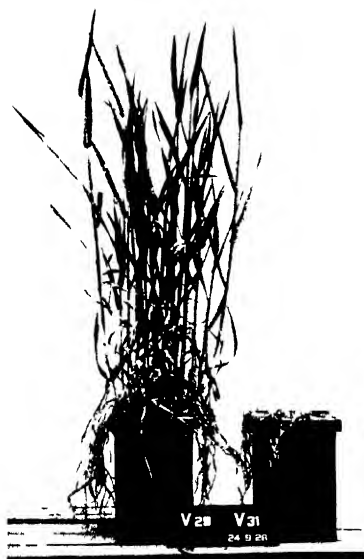


Fig. 3.



Fig. 4





Fig. 3.

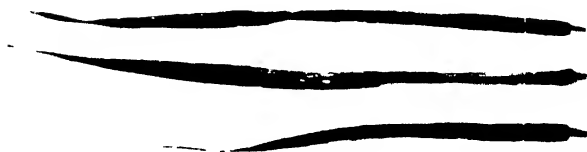


Fig. 2.



Fig. 1.



## A MOSAIC VIRUS OF GRASSES, NOT VIRULENT TO SUGAR CANE

By H. H. STOREY, M.A., PH.D.

(*East African Agricultural Research Station, Amani.*)

THE leaf-mottling, typical of a mosaic disease, is commonly to be found in a number of grass species when growing in the vicinity of mosaic-diseased sugar cane (*Saccharum officinarum* L.). This condition has generally been assumed to be due to the cross-transmission of a single virus between the several hosts. There is experimental support for this view. Brandes<sup>(2)</sup>, Kunkel<sup>(4)</sup>, Hadden<sup>(3)</sup> and others successfully produced the mosaic pattern in healthy plants of sugar cane, maize (*Zea Mays* L.), *Sorghum* spp. and other grasses by experimental transmission of a virus from sugar cane and others of these hosts. In consequence it has generally been supposed that all typically mosaic-diseased grasses were affected by a common virus and were reservoirs whence the virus might be carried to sugar cane.

In the course of a search for wild host-plants of the sugar cane mosaic virus in South Africa, I encountered evidence which threw doubt upon this conjecture. Whereas I had no reason to suppose that the mosaic-diseased grasses in the sugar cane area of Natal were not carrying the sugar cane virus—and experiments later showed that the virus might be transmitted from certain of them to sugar cane—yet I found a mosaic widespread in maize and *Sorghum* spp. in the Transvaal where no mosaic-diseased sugar cane was known to exist.

This Transvaal virus produced signs in maize and *Sorghums* indistinguishable from that caused by the sugar cane virus in these hosts. Nevertheless my experiments led me to believe that this virus is incapable of producing any visible effect upon sugar cane. I have stated this conclusion in a previous brief report<sup>(7)</sup>. I now amplify the details of the evidence upon which it is based.

This work was carried out at the Natal Herbarium, Durban, under the direction of Dr I. B. Pole Evans. I acknowledge the assistance of J. S. Mackay and R. F. W. Nichols.

## FIELD OBSERVATIONS.

The Transvaal mosaic was first observed in 1924 in plants of the wild grass species, *Sorghum arundinaceum* Stapf<sup>1</sup>, which had been collected in the neighbourhood of the Groenkloof Experiment Station, near Pretoria, and had been planted in a plot in this station. In subsequent years the disease was evident in this plot and in a second plot arising from roots transplanted to the Prinshof Experiment Station, Pretoria. Diseased plants of this species were not found elsewhere in the Transvaal, but no considerable search for them was made. A mosaic was, however, found in maize and cultivated Sorghums, both of the grain and sweet types, on many farms in the Rustenburg and Waterberg districts of the Transvaal. In these areas a number of small plots of sugar cane, of varieties known to be susceptible to mosaic, were at this time all found to be free from mosaic disease<sup>2</sup>.

Although I have not demonstrated the identity of the viruses causing the mosaics in *Sorghum arundinaceum* at Pretoria and in maize and cultivated Sorghums in the neighbouring Rustenburg and Waterberg regions, yet I have no reason to doubt their identity. My experiments in cross-transmission were, however, performed with *Sorghum arundinaceum* only.

In Natal meanwhile a mosaic of sugar cane and a number of grasses was prevalent and was spreading freely to susceptible varieties of sugar cane. I have recorded my observations upon this mosaic in an earlier paper(5). For reasons there stated I believe this disease to have been introduced into Natal within recent years in cane sets imported from the New World, and therefore to be identical with the sugar cane mosaic known in most cane-growing countries.

## SIGNS OF THE DISEASE.

The signs of the common sugar cane mosaic in sugar cane, maize and Sorghums are well known and have been frequently described (e.g. (1), (4), (5)). To these the signs of the Transvaal mosaic in the several hosts have closely conformed, and I have found no visible character for separating it from the common mosaic, as shown by affected plants

<sup>1</sup> *Sorghum arundinaceum* Stapf. Determined by Miss Stent, National Herbarium, Pretoria. A weak tufted perennial under South African conditions. Described by Stapf as an annual in *The Flora of Tropical Africa*, ix, Pt. 1, p. 114, and originally included by him with *S. verticilliflorum* in *Andropogon halepensis* var. *effusus* (Flor. Cap. vii, 346).

<sup>2</sup> A recent unconfirmed report records mosaic in a plot of cane at Warmbaths, Transvaal.



in Natal. The tendency, observed by Brandes<sup>(1)</sup>, for the mosaic pattern to become suppressed as the leaves age was evident in maize and Sorghums when affected with either virus. Indeed in affected *Sorghum arundinaceum* plants the mosaic pattern was often barely visible even on the youngest leaves. On the other hand in the field in the Waterberg maize frequently exhibited an unusually pronounced contrast between the light and dark green areas of the mosaic-affected leaf. This deviation from the usual type was not shown by maize plants of the Hickory King variety when experimentally infected with the Transvaal virus taken from *Sorghum arundinaceum*. These plants were indistinguishable from those infected with the Natal virus. While this evidence might be held to indicate that the Waterberg maize virus was distinct from the Pretoria Sorghum virus, it is quite insufficient to prove this point. I consider it to be more probable that climatic conditions or varietal differences account for the pronounced manifestations observed in the Waterberg.

#### EXPERIMENTAL METHODS AND RESULTS.

*Cage experiments.* Experiments were carried out in an insect-proof greenhouse to determine whether *Aphis maidis* Fitch, the known vector of sugar cane mosaic, could transmit the Transvaal mosaic. Groups of aphides were caged upon single leaves of the experimental plants, by means of the glass tubes described elsewhere<sup>(6)</sup>. Mosaic was thus successfully transmitted to maize by aphides collected on diseased maize,

Table I.

#### *Transvaal mosaic.*

Tests for transmission to maize, by *Aphis maidis*. Glass-tube leaf-cage method in greenhouse, 6–12 aphides to each plant. Mosaic appeared in 8–30 days.

Date	Source of infection	Tests on maize		Controls		Details
		No.	Diseased	No.	Diseased	
11. ii. 25– 23. iv. 25	Mosaic maize	12	10	12	Nil	Aphides tested immediately after collection upon diseased plants in the field in the Transvaal
	Mosaic cultd. Sorghum	8	7	8	Nil	
	Mosaic, <i>Sorghum arundinaceum</i> *	15	4	15	Nil	
26. vi. 25– 20. x. 25	Mosaic, <i>Sorghum arundinaceum</i>	30	12	30	Nil	Non-infective aphides from cultures on healthy plants fed for a time upon the diseased Sorghum plants

\* Aphides taken from diseased plants in the Groenkloof and Prinshof experiment stations, Pretoria.

## 528 *Mosaic Virus of Grasses, not Virulent to Sugar Cane.*

cultivated Sorghums and *Sorghum arundinaceum* (Table I). Aphides, which were previously non-infective but were fed for a period upon a diseased *Sorghum arundinaceum* plant, were also able to transfer the mosaic to maize.

For experiments in transmission to sugar cane, large cages were constructed at Durban, Natal, covering an area of 8 feet square, with glass roofs and wire-gauze sides. The experimental cane plants were raised from sets in tins within the cages, together usually with a number of maize seedlings. Diseased plants, of the kind to be used as the source of infection, were placed between the experimental plants. When all plants were through the ground and growing rapidly, large numbers of *Aphis maidis* were distributed upon the diseased plants. After a period of feeding and development on the diseased plants, the aphides moved naturally to the healthy ones, upon which they were usually to be observed feeding. Aphid-distribution was repeated several times. The experimental plants were retained under observation in the cages for about three to five months.

An adjacent cage was occupied by control plants, similarly treated except that there were no diseased plants and no aphides were distributed. The most important function of these controls was to ensure that no cane sets were already carrying the mosaic virus at the time of planting; consequently at least one control set was taken, usually from the top, from each whole cane, which was cut into the sets which provided the experimental plants. During these experiments no control plant developed mosaic.

Aphides for distribution in the whole series of experiments were reared upon healthy *Sorghum* seedlings. They were all the progeny of one original non-infective culture. At intervals samples of the aphides were removed from the culture cages and tested for infective power by caging on maize or cane. In five tests all of the plants remained free from mosaic disease<sup>1</sup>.

Experiments in the transmission of the Natal virus were thus carried out, the sources of infection being sugar cane, *Sorghum arundinaceum* and *Setaria sulcata* Raddi, all diseased specimens collected in the field in the Natal cane area. No experiment failed to give some positive infections of sugar cane, although the infections occurred with that

<sup>1</sup> The tests were as follows: On 9. x. 25 groups of aphides tubed on 8 maize plants; on 23. iii. 26 caged with 30 maize plants; on 1. vii. 26 caged with 29 maize plants; on 17. xii. 26 caged with 41 cane plants; on 14. iv. 27 caged with 44 cane and 62 maize plants. All plants remained free from mosaic disease.

irregularity which is commonly experienced in experiments in mosaic transmissions (Table II).

Table II.

*Natal mosaic.*

Large cage experiments in transmission to sugar cane and maize. Cane varieties infected—Port Mackay, Rose and Striped Bamboo, Gingor, P.O.J. 213, D. 1135, Q. 813, 1900 seedling, Co. 210, Co. 213, and four varieties of uncertain relations.

Date	Source of infection	Cane plants exposed		Maize plants exposed		Control cane plants	
		No.	Diseased	No.	Diseased	No.	Diseased
1. vii. 26	Mosaic cane, variety P.O.J. 213	77	30	44	10	52	Nil
17. xii. 26	Mosaic cane, variety Rose Bamboo	15	6	—	—	37	Nil
14. v. 27	Mosaic cane, several varieties	52	43	—	—	80	Nil
9. i. 26	Mosaic <i>Setaria sulcata</i>	42	12	24	1	36	Nil
14. iv. 27	Mosaic <i>Setaria sulcata</i>	25	6	—	—	36	Nil
25. xii. 25	Mosaic <i>Sorghum arundinaceum</i>	72	12	48	48	30	Nil

Experiments designed to transmit the Transvaal mosaic to sugar cane were performed in a similar manner, employing the same strain of aphides. The source of infection was diseased *Sorghum arundinaceum* transplanted from Pretoria. In two experiments, the results of which are summarised in Table III, all of 100 cane plants, of varieties known to be susceptible to the Natal virus, remained free from all signs of mosaic. Meanwhile mosaic developed in each experiment in maize seedlings exposed, and in one in *Sorghum arundinaceum* seedlings exposed.

Table III.

*Transvaal mosaic.*

Large cage experiments in attempted transmission to sugar cane, maize and *Sorghum arundinaceum*. Cane varieties used: Rose Bamboo, Port Mackay, Black Innes, D. 1135, 1900 seedling, Q. 813. Maize and *Sorghum* seedlings raised in cage from seed.

Date	Source of infection	Cane plants exposed		Maize plants exposed		<i>Sorghum arundinaceum</i> plants exposed		Controls	Remarks
		No.	Diseased	No.	Diseased	No.	Diseased		
9. x. 25	Mosaic <i>Sorghum arundinaceum</i>	56	Nil	80	17	15	11	36 cane plants—healthy. 15 <i>Sorghum</i> plants—healthy	Aphides observed feeding on all cane plants
26. iii. 26	Mosaic <i>Sorghum arundinaceum</i>	44	Nil	15	11	—	—	42 cane plants—healthy	Aphides observed feeding on many cane plants

## 530 *Mosaic Virus of Grasses, not Virulent to Sugar Cane*

*Field experiment.* A field experiment with the Transvaal mosaic was carried out at the Prinshof Experiment Station, Pretoria. In December, 1925, a plot was planted with about 50 sets each of five cane varieties known to be susceptible to sugar cane mosaic (D. 1135, Port Mackay, Rose Bamboo, Striped Bamboo, Black Innes), and alongside was planted a plot of mosaic-diseased *Sorghum arundinaceum* taken from the Groenkloof Experiment Station. On the adjacent land also were sown plots of maize, grain Sorghum and sweet Sorghum. During the following months the last plots all developed mosaic in a large proportion of the plants. Nevertheless the cane remained free from mosaic-symptoms, and ratoons in the seasons 1926-7 and 1927-8, still alongside the ratoons from the diseased Sorghum roots, were similarly healthy. During each season many colonies of *Aphis maidis* were to be seen upon the diseased *Sorghum arundinaceum* plants.

### DISCUSSION.

In this paper I endeavour to prove that the Transvaal virus is not virulent to sugar cane, but the negative evidence required for a proof of this kind is generally not easily obtained. Any experiment, purporting to demonstrate the immunity of a plant species to a disease, must meet the charge that suitable conditions for infection have not been provided.

I believe that my evidence for the immunity of sugar cane to the Transvaal mosaic will withstand this charge. With a virus disease the most trustworthy proof of the immunity of a plant species is afforded by its healthy growth in a region where the disease is known to spread readily to susceptible species. The Pretoria experiment provided evidence of this kind. Some 250 cane plants survived uninfected through three seasons, although maize and Sorghums alongside became diseased. The conditions of this trial were particularly rigorous, for the adjacent diseased Sorghum was in each season colonised by *Aphis maidis*, individuals of which were able to infect maize in my experiments. It is hardly conceivable that the cane plants were not frequently subjected to the feeding of aphides carrying the virus derived from the diseased Sorghum.

The healthy condition of the cane in the farm plots seen in the Transvaal affords some confirmatory evidence for its immunity to the mosaic of this region. This evidence is, however, not worth much since the cane might have accidentally escaped infestation by virus-bearing aphides. Similarly the possible recent occurrence of mosaic in Transvaal cane weighs little against my hypothesis, since mosaic-infected cane sets could have been carried from Natal into the Transvaal.

It might be argued, however, that the conditions in the Transvaal, though favourable to the infection of maize and Sorghums, were yet in some way unfavourable to the infection of cane. Conceivably the Transvaal race of aphides was incapable of feeding on cane. For this reason experiments were carried out at Durban, observing a technique which maintained, as nearly as was possible in a region where the cane mosaic was endemic, the conditions holding in a field trial. Furthermore, this method was always successful in the transmission of the Natal mosaic to cane, including one experiment where the source of the Natal virus was *Sorghum arundinaceum*. Nevertheless in two separate experiments all of one hundred cane plants resisted infection by the Transvaal virus from *Sorghum arundinaceum*. It is certain that the majority of the cane plants were fed upon by aphides, of a strain capable of infecting cane with the Natal virus, and actually carrying a virus virulent to the adjacent maize and Sorghum.

I therefore conclude that the Transvaal virus is not virulent to sugar cane, or at least, that it is incapable of producing the signs of mosaic disease in sugar cane. This conclusion denotes some difference in the Transvaal virus from the Natal (or common sugar cane) virus. Already several virus diseases of grasses have been recognised, separable by their signs and sometimes by their insect-vectors; as, for example, sugar cane mosaic transmitted by *Aphis maidis* and streak transmitted by *Balclutha* (*Cicadulina*) *mbila* Naude. Here, however, there is a definite difference of kind. The difference in the mosaics which I have studied is one of degree, of virulence to a range of host-plants.

#### SUMMARY.

This paper records studies of a mosaic disease observed in maize and *Sorghum* spp. in the Transvaal, South Africa. In these hosts the signs produced were not distinguishable from those produced by the common sugar cane mosaic virus. Leaf-cage experiments showed that *Aphis maidis* Fitch was capable of transmitting the virus to maize.

In the part of the Transvaal where the mosaic was found all sugar cane seen was free from mosaic. Sugar cane failed to contract the disease, both in a field experiment extending over three years in the Transvaal and in large cage experiments in Natal. The method employed in the Natal experiments was always successful in transmitting mosaic to sugar cane when the source of infection was either diseased cane or diseased grasses collected in the neighbourhood of diseased cane.

## 532 *Mosaic Virus of Grasses, not Virulent to Sugar Cane*

It is concluded that the Transvaal virus is not virulent to sugar cane and is therefore different from the common sugar cane mosaic virus.

### REFERENCES.

- (1) BRANDES, E. W. (1920). Mosaic disease of corn. *Journ. Agric. Research*, XIX, 517-21.
- (2) BRANDES, E. W. and KLAPHAAK, P. J. (1923). Cultivated and wild hosts of sugar-cane or grass mosaic. *Ibid.* XXIV, 247-62.
- (3) HADDEN, F. C. (1928). Sugar cane mosaic and insects. [*Hawaiian Planters' Record*, XXXII]; *Facts About Sugar*, XXIII, 758-62.
- (4) KUNKEL, L. O. (1924). Studies on the mosaic of sugar cane. *Bull. Exp. Sta. Hawaiian Sugar Planters' Association, Bot. Ser.* III, 115-67.
- (5) STOREY, H. H. (1924). Diseases of sugar-cane of the mosaic type in South Africa. Part I. *Journ. Dept. Agric. South Africa*, IX, 108-17.
- (6) — (1925). The transmission of streak disease of maize by the leafhopper *Balclutha mbila* Naude. *Ann. Appl. Biol.* XII, 422-39.
- (7) — (1927). Strains of the viruses affecting the Gramineae. *Proc. 2nd Conf. Int. Soc. Sugar Cane Technologists, Havana, Cuba*, pp. 87-8.

(Received April 12th, 1929.)

# OBSERVATIONS DURING 1927-28 ON THE INCIDENCE OF "RUSTS" ON VARIOUS SELECTED WHEAT VARIETIES, WITH SPECIAL REFERENCE TO THE INTENSITY OF YELLOW RUST, *PUCCINIA GLUMARUM*, ERIKS. AND HENN.

By W. A. R. DILLON WESTON, M.A.

(School of Agriculture, Cambridge.)

THE following observations have been made on the Cambridge University Farm on wheat varieties grown for the purpose of testing their resistance to *Tilletia caries*. In each case the seed, prior to sowing, had been very heavily contaminated at approximately the same rate with bunt spores. The size of the plots, varied but in any one set of experiments was constant, and was never less than 18 feet of drill. One hundred leaves from each variety were examined in the laboratory for the presence of rust and recorded as having none, slight, moderate, or severe. A leaf was recorded as severely rusted if the whole or  $\frac{3}{4}$  of its surface was completely yellow with pustules, moderate if  $\frac{1}{4}$  to below  $\frac{3}{4}$  rusted, slight if from a few scattered pustules or streaks to below  $\frac{1}{4}$  rusted. The number of leaves showing a moderate attack was added to the number showing a severe attack, the total expressed as a percentage of the whole sample (100 leaves) was taken, in what follows, as the "Intensity of yellow rust attack." This system of estimation was the same as that described recently (1), but unless otherwise stated the leaves were taken at random from clean and bunted plants; in some cases, however, separate estimations were given of the rust intensity on clean and bunted tillers. In such a crude classification the main difficulty was in determining whether a leaf was or was not attacked, since with many varieties there were peculiar fleckings on the leaf, and, on these, pustules of yellow rust might or might not appear. In some cases these fleckings merged into each other until the leaf was almost completely yellow, when the yellowing must have had much the same effect on the plant as a moderately bad attack of yellow rust, since photosynthesis on those leaves could not take place. It appears that flecks of this description may be caused by the failure of the pathogen to establish itself on the host (2). No estimations were made of brown rust, *Puccinia triticina*, or black rust, *P. graminis*,

## 534 Incidence of "Rusts" on various Wheat Varieties

but some observations were recorded. It is difficult to tell if a variety markedly susceptible to yellow rust is susceptible or resistant to brown rust since the latter has little or no chance of attacking it. The percentage of bunt in these varieties was also recorded, and this was estimated at harvest by taking a total head count or by harvesting 1000 ears at random.

In addition to the varieties mentioned in the tables a number of other varieties were also tested for their resistance or susceptibility to rust and bunt. These have included sixteen Australian varieties, Felix, Maharajah, Sultan, Rajah, President, Sirdar, Bearded Gluyas, Late Gluyas, Gluyas, King's White, King's Early, King's Red, Waratah, American 8, Warden, Canberra (all *T. vulgare* Host.). With the exception of Warden none showed very marked resistance to *P. glumarum*. The yellow rust intensity of this variety on July 10th was 8 and the bunt percentage was 20. The nearest approach to this figure was Canberra with a rust intensity of 40 and a bunt percentage of 43. In this test the leaves were taken at random from clean and bunted tillers.

With these varieties it was interesting to note the rapid spread of yellow rust between June 28th and July 10th. Prior to June 28th a number of these varieties were free from rust, but after July 10th with the exception of these two, they had a rust intensity of 100.

Nineteen varieties resistant in America to strains of brown rust *P. triticina* were also tested, they included Warden, Hope, Chinese, Resaca, Malakoff, Hussar, Norka, Democrat, Mediterranean, Webster and Fultz (two selections). These varieties are all *T. vulgare* Host. Brown rust was not observed on any of these, but black rust was noted on Resaca, Hussar, Democrat, Mediterranean and Fultz. Yellow rust was observed on all the above varieties and the intensity was high. Warden on July 3rd had a rust intensity of 2, but this increased until July 23rd when the rust intensity was 90; it should, however, be stated that the bunt percentage was 80. Democrat and Mediterranean showed some resistance to yellow rust; in the latter case the rust intensity on June 17th was 0, but on July 25th it was 60, and the bunt percentage was 63.

In examining Table I it is of interest to note the following facts. With the exception of Marshal Foch, *T. vulgare* Host., there is a marked resistance to *P. glumarum*. This is especially noted in (B), *T. sphaerococcum* Perc., (D), *T. durum* Desf., American Club, *T. compactum* Host., Rivet, *T. turgidum* L. and (F), *T. monococcum* L. The resistance of *T. monococcum*, *T. durum* and *T. compactum* appears to be complete. The resistance of Red Fife, *T. vulgare* Host., (E), *T. polonicum* L., (C),



*T. spelta* L. and Persian Black, *T. persicum* Vav., is less marked. It is of interest to note that *T. monococcum* with 14 chromosomes shows complete resistance to the common wheat pathogens, but it will be seen that almost complete resistance to one pathogen *P. glumarum* is also shown by *T. compactum* and *T. sphaerococcum*, 42 chromosomes; and *T. durum* and *T. turgidum*, 28 chromosomes. In estimating the resistance of a variety to yellow rust it is an excellent criterion to note the reaction of the variety to *P. glumarum* when it is bunted. The writer considers that true and false resistance may be estimated in this way. For example, American Club when bunted showed *no rust at all on the leaves*, although it was interesting to observe that there was very slight rust on the bunted ears.

Dr E. F. Gaines, from whom the varieties on Table II were received, states *in litt.*: "These varieties have been bunt free at Pullman, Washington, in 1927. We find Hope and Khapli to be resistant to stem and leaf rust, and according to field observations they are probably resistant to stripe rust, *P. glumarum*." These wheats were tested in triplicate plots, but one set of results only is given, since the second and third sets confirm the first.

In Table III Iumello, Marquillo (Ma  $\times$  Turkey 11 : 21 : 27), Marquillo 6887, and Marquillo (Ma  $\times$  Turkey 11 : 21 : 22) appear to be markedly resistant to *P. glumarum*.

Kota is slightly more susceptible to yellow rust than Marquis, and the hybrid from these (Ceres) is as susceptible as Kota. Of the four *durum* varieties, Iumello and Pentad show some marked resistance to yellow rust, but Iumello is the more resistant to both bunt and rust.

The *diococcum* varieties, Vernal Emmer and Khapli, had a rust intensity on July 11th of 100; Khapli was literally yellow with rust pustules and suffered the worst attack of any of the varieties that were grown in 1928, the intensity being higher than that which occurs on White Odessa when it is grown in this country. The *durum* and *diococcum* races are in the "Emmer" group and have the 28 chromosome number, the remainder of the above wheats are *vulgare* and have 42 chromosomes.

#### ENGLISH VARIETIES.

Nineteen English varieties were tested for their resistance to yellow, brown and black rust and also to bunt. These varieties included Benefactor, Little Joss, Marshal Foch, Rector, Wilhelmina, Harvester Red and White Wonder. Leaves were examined from clean and bunted tillers. Rivet and Little Joss showed marked resistance when clean, but when

Table I.

Showing the intensity of yellow rust on certain races of wheat.

Variety	Date of observation	% of yellow rust					Moderate + severe	% of bunted ears	Remarks
		None	Slight	Moderate	Severe				
(A) <i>Triticum diococcum</i> Schubl. ...	... 28. vi. 28	3	59	38	0	38	37	37	Rust. Intensity when bunted, 100
(B) <i>T. sphaerococcum</i> Perc.	... 15. vii. 28	No yellow rust observed.	Yellow lesions on leaves					20	Black rust observed moderately bad. More yellow lesions on leaves when bunted
(C) <i>T. spelta</i> L. Red Fife, <i>T. vulgare</i> Host.	... 28. vi. 28	23	43	32	2	34	2	2	Brown rust not observed
	... 28. vi. 28	49	47	0	4	4	25	25	Bunted ears have more yellow rust. Brown rust observed. Rust intensity when bunted 50
(D) <i>T. durum</i> Desf.	... 28. vi. 28	100	2	0	0	0	0	0	Brown rust, occasional pustules. Yellow rust or bunted ears
Persian Black, <i>T. persicum</i> Vav.	... 28. vi. 28	Rust very slight but much yellowing. Rust pustules not active					25	25	Ergot observed. Brown rust slight to moderate. 31. vii. 28
American Club, <i>T. compactum</i> Host.	—	Rust not observed					57	57	Brown rust bad. Rust intensity of leaves when bunted 0. Yellow rust or bunted ears
Marshal Foch, <i>T. vulgare</i> Host.	... 28. vi. 28 10. vii. 28	6 0	67 0	27 84	0 16	27 100	37	37	Brown rust observed. Rust intensity when bunted 100
Rivet, <i>T. turgidum</i> L.	... 28. vi. 28 10. vii. 28	93 82	7 18	0 0	0 0	0 0	56	—	Rust intensity when bunted 18. Brown rust observed
(E) <i>T. polonicum</i> L.	... 28. vi. 28 10. vii. 28	7 54	65 38	28 8	0 0	28 8	12	—	Rust intensity when bunted 80. Brown rust not observed
(F) <i>T. monococcum</i> L.	... —	0	0	0	0	0	0	0	No brown rust. No black rust observed

Table II.  
*Showing intensity of T. caries and P. glumarum on certain American spring wheat varieties.*

Variety	C.I. no. Row no.	Date of observa- tion	% of yellow rust					Remarks
			None	Slight	Moderate	Severe	Moderate + severe	% of bunted ears
Hope, <i>Triticum vulgare</i> Host.	341	20. vi. 28	2	29	57	12	69	2
		29. vi. 28	5	0	7	38	90	—
		10. vii. 28	—	—	—	—	100	—
Quality, <i>T. vulgare</i> Host.	325	20. vi. 28	89	6	5	0	5	4
		29. vi. 28	43	6	1	0	2	—
		10. vii. 28	60	40	0	0	0	—
Alaska, <i>T. turgidum</i> L.	308	20. vi. 28	40	33	27	70	27	12
		11. vii. 28	—	50	50	—	50	—
Khapli, <i>T. dicoccum</i> Schubl.	310	20. vi. 28	3	3	16	78	94	26
		11. vii. 28	—	—	—	—	100	—
Marquis × Turkey, <i>T. vulgare</i> Host.	—	20. vi. 28	3	57	35	5	40	31
		11. vii. 28	0	0	30	70	100	—

Table III.

*Showing the intensity of T. caries and P. glumarum on certain wheat varieties resistant in America to biologic races of P. graminis.*

Variety	Cl. no. Row no.	Date of observ- ation	% of yellow rust					% of bunted ears	Remarks
			None	Slight	Moderate	Severe	Moderate + Severe		
Ceres, <i>Triticum vulgare</i> Host.	6900	19. vi. 28 21. vii. 28	29	47	25	9	34 100	31	<i>Ustilago tritici</i> observed. No black rust observed
Mindum, <i>T. durum</i> Desf.	5296	19. vi. 28 21. vii. 28	32	35	24	11	35 100	24	Yellow rust very bad on ears. No black rust
Iumello, <i>T. durum</i> Desf.	1736	19. vi. 28 21. vii. 28	94	6	—	—	0	6	Yellowing of foliage. No brown rust observed. No black rust observed
Pentad, <i>T. durum</i> Desf.	3322	19. vi. 28	62	26	12	0	12	80	Yellowing of foliage. No brown rust observed. No black rust observed
Webster, <i>T. vulgare</i> Host.	3780	19. vi. 28 29. vi. 28 3. vii. 28 11. vii. 28	8 0 0 —	30 11 5 —	44 21 9 —	18 18 36 —	62 78 90 100	73 — — —	No black rust observed
Marquis, <i>T. vulgare</i> Host.	3671	19. vi. 28 29. vi. 28 11. vii. 28	42 3 10	50 12 30	8 22 35	0 3 25	8 50 60	6 — —	Mildew noticeable. No black rust. Rust in- tensity of bunted plants 80
Vernal Emmer, <i>T. diococcum</i> Schubl.	3686	19. vi. 28 29. vi. 28 11. vii. 28	68 11 —	15 12 —	12 18 —	5 9 —	17 54 100	23 — —	Mildew noticeable. No black rust

Khapi, <i>T. diococcum</i> Schubl. ...	4013	11. vii. 28	—	—	—	100	—	Strongly susceptible to bunt. No black rust
Velvet Don, <i>T. durum</i> Desf. ...	1445	19. vi. 28 29. vi. 28 11. vii. 28	63 0 0	25 18 30	12 22 70	0 10 0	46	Rust intensity of bunted plants 100. No brown rust. No black rust
Kota, <i>T. vulgare</i> Host. ...	5878	19. vi. 28 29. vi. 28 11. vii. 28	96 31 0	4 19 0	0 0 40	0 0 30	69	Mildew noticeable. Rust intensity and yellowing of bunted plants 100. No black rust
Marquillo, <i>T. vulgare</i> Host. ...	11. 21. 27	19. vi. 28 29. vi. 28 10. vii. 28	48	2	Not observed 0 Yellowing	0	42	Mildew noticeable. Rust intensity of bunted plants 74. No black rust. No brown rust
Marquillo, <i>T. vulgare</i> Host. ...	6887	29. vi. 28	Occasional pustules of yellow rust					Ergot observed. No brown rust observed. No black rust
Progress, <i>T. vulgare</i> Host. ...	6902	19. vi. 28 29. vi. 28 11. vii. 28	36 25 0	62 25 0	2 0 60	0 0 40	82	No brown rust observed. No black rust
Reliance, <i>T. vulgare</i> Host. ...	7370	19. vi. 28 29. vi. 28 11. vii. 28	10 0 —	51 0 —	36 3 —	3 47 —	15	No black rust
Marquillo, <i>T. vulgare</i> Host. ...	11. 21. 22	19. vi. 28 10. vii. 28	— 48	— 52	— 0	— 0	20	No rust noted on ears. Brown rust observed very slight. Brown neck. No black rust
Hope, <i>T. vulgare</i> Host. ...	8118	19. vi. 28 29. vi. 28 11. vii. 28	23 6 —	53 0 —	24 24 —	0 20 —	2	No brown rust. No black rust

## 540 Incidence of "Rusts" on various Wheat Varieties

bunted were susceptible, Rivet being less susceptible when bunted than Little Joss. Brown and black rust was observed on each of the 19 varieties, black rust was diagnosed in the teleutospore stage and the observations on this rust were made on August 14th. Brown rust generally develops extensively in East Anglia in the second or third week of June, it occurs too late to do serious damage. Black rust, from our own observations, on the University Farm occurs later still; in 1928 it was observed at the end of July, and on August 14th the teleutospore stage was observed on all the English varieties tested, but no estimations of its intensity were made.

### DISCUSSION.

Sax (3) concludes that there is a relationship between the chromosome number of the wheat races and their resistance or susceptibility to pathogens. He considers that the races with the lower chromosome number are more resistant than races of a higher number. He states: "The percentage of infection (*i.e.* bunt) in *T. polonicum* was 8, in *T. diococcum* 10, in *T. durum* 29, in *T. turgidum* 33. In the vulgare group *T. spelta* was comparatively resistant with only 10 per cent. infection, but the percentage of bunt found in *T. vulgare* was 70 and in *T. compactum* it was 64 per cent. In general, the species and varieties of wheat which are resistant to rust are resistant to bunt and *vice versa*. It is probable, then, that varieties found to be resistant to rust in the middle west would be valuable disease-resistant varieties for the Pacific coast where bunt is the most important cereal disease. Likewise the valuable results in breeding bunt-resistant wheats on the Pacific coast could be utilised in regions where rust is prevalent. . . . With an increase in chromosome numbers, 14-28-42, there is an increase in variability and adaptability, and increased susceptibility to rust, mildew and bunt, a better quality of gluten in the grain, and the economic value is greater. . . . Rust and bunt resistance apparently depend on the same factors, so that results in breeding wheats resistant to rust can be applied to bunt and *vice versa*. . . ."

From our own observations we would not accept these broad generalisations, for although it seemed clear that *T. monococcum* with its 14 chromosomes showed complete resistance to the common wheat pathogens, the 28 chromosome group when compared with the 42 group varied considerably in the relative resistance to disease organisms.

*The races of wheat quoted by Sax were not contaminated with their own bunt, and the writer has shown (4) that when this is done resistant varieties*

become susceptible. The writer considers that there is no firm foundation for believing that the species and varieties of wheat which are resistant to rust are resistant to bunt and *vice versa*. For is not American Club (*T. compactum*) remarkably resistant to *P. glumarum* even when bunted, yet is it not markedly susceptible to *T. caries*? Again, Rivet (*T. turgidum*) although highly resistant to *P. glumarum* is markedly susceptible to *T. caries*, and White Odessa (*T. vulgare*) that is markedly resistant to *T. caries* (under certain conditions) is 100 per cent. susceptible to *P. glumarum*.

#### SUMMARY.

1. Observations on the incidence of yellow, brown and black rust on selected wheat varieties are recorded.
2. The intensity of yellow rust on these varieties is given.
3. It is shown that bunt increases the susceptibility of varieties to yellow rust.
4. It is suggested that in testing wheat varieties for resistance to *P. glumarum* the seed should be previously bunted, and that if no yellow rust then appears that the variety should be regarded as truly resistant.
5. It is suggested that a generalisation cannot be made that the 28 chromosome group is more resistant to pathogens than the 42 chromosome group, and also that there is no firm foundation for believing that the species and varieties of wheat which are resistant to rust are resistant to bunt and *vice versa*.

In concluding these observations the writer expresses his thanks to Dr E. F. Gaines, Pullman, Washington, Prof. E. C. Stakman, St Paul, Minnesota, and Dr Mains, Perdue University, Indiana, for supplying him with a number of American wheat varieties, also to Mr F. T. Brooks, Cambridge, for his valued criticism.

#### REFERENCES.

- (1) DILLON WESTON, W. A. R. (Feb. 1927). *Ann. App. Biol.*
- (2) MARRYAT, D. C. E. (1907-8). *Journ. Agric. Sci.* xi.
- (3) SAX, K. (1923). *Genetics*, viii, No. 1.
- (4) DILLON WESTON, W. A. R. (Feb. 15, 1929). *Nature*.

(Received February 19th, 1929.)

# TREATMENT OF SUGAR BEET "SEED" TO PREVENT SEEDLING DISEASES

BY R. C. WOODWARD, B.Sc., PH.D.

*(School of Rural Economy, Oxford),*

AND W. A. R. DILLON WESTON, M.A.

*(School of Agriculture, Cambridge).*

(With Plate XXV and 3 Text-figures.)

## CONTENTS.

	PAGE
I. THE ORGANISMS RESPONSIBLE FOR BLACKLEG IN SUGAR BEET SEEDLINGS . . . . .	543
II. SOME CONSIDERATIONS OF DAMAGE . . . . .	544
III. PRELIMINARY TRIALS WITH DISINFECTANT "SEED" TREATMENTS . . . . .	545
(a) Trials, 1926 . . . . .	545
(b) Greenhouse trials, 1928 . . . . .	545
(c) Preliminary field trials, 1928 . . . . .	546
(d) Analysis of all seedlings above and below soil level . . . . .	548
(e) Is the beneficial effect of wet treatment due to the influence of water or the chemical? . . . . .	549
(f) Estimations in the laboratory of the percentage of diseased growths which arose from treated and untreated "seed" . . . . .	550
(g) Preliminary trials on the treatment of "seed" with sulphuric acid and comparisons with treatment with the mercury material . . . . .	551
IV. COMPARATIVE FIELD TRIALS WITH THREE "SEED" TREATMENTS . . . . .	553
(a) Field trials to estimate the "plant" establishment and percentage of Blackleg as the result of three "seed" treatments . . . . .	553
(b) Vigour of the plants as judged by green and dry weights of seedlings derived from the treated and untreated samples . . . . .	554
(c) Effect of the treatments on yield and the percentage of sugar . . . . .	555
V. EFFECT OF THE TREATMENTS ON COMMERCIAL "SEED" AS SHOWN BY THE RESULTS OF TRIALS IN WHICH TEN DIFFERENT VARIETIES OF "SEED" WERE TESTED . . . . .	558
(a) Effect on germination . . . . .	558
(b) Effect on "plant" establishment . . . . .	559
(c) The relationship of the percentage of germination of the "seed" and percentage of germinated seed-clusters producing diseased growths to the mean percentage of increase in the number of plants in response to treatment . . . . .	561
(d) Preliminary observations relating to commercially supplied "seed" treated with the mercury material . . . . .	562
VI. DISCUSSION . . . . .	564
VII. SUMMARY . . . . .	565
EXPLANATION OF PLATE . . . . .	566



SUGAR beet "seed," as will be seen, is seldom free from disease, and it is for this reason that certain continental countries have resorted to "seed" disinfection. In England, however, since the positive or negative value of "seed" treatments for this purpose had not been tested it was thought that an investigation would be desirable. Further, since sugar beet is already an important crop in the Eastern and South Midland Advisory Provinces it was manifest that in such an investigation would be offered a local problem of first magnitude and importance.

#### I. THE ORGANISMS RESPONSIBLE FOR BLACKLEG IN SUGAR BEET SEEDLINGS.

It is not proposed to describe the fungi responsible for infection of "seed" but rather to show the value, or otherwise, of certain methods which have been adopted for the disinfection of the "seed." The fungus which infects the "seed," and which is said to be mainly responsible for a poor "plant" and death of seedlings in the field, is *Phoma Betae*, the symptoms produced being commonly described as Blackleg. The identity of this fungus was confirmed in 1927 when cultures made from English sugar beet seedlings were compared with cultures of *Phoma Betae* from Dutch seedlings and were found to be identical. Although there are other fungi, such as *Pythium de Baryanum* and *Aphanomyces laevis*, both of which as inhabitants of the soil may cause Blackleg, the general symptoms produced on the young seedlings by these organisms are thought to be similar and are first exemplified by water-soaked spots on the young stem at, or just below, soil level; the lesions so made increase in size, become brown, then black in colour and, by finally cutting off the water channels, cause rapid wilting and death. If such plants are carefully dug up and examined, a black constriction at soil level is apparent, and generally the young infected root presents a characteristically threadlike appearance. If the plant is older and more vigorous when attacked it may recover; in this case the diseased tissue may be confined to the outer layers until it is finally thrown off, leaving a typical scar.

Attack by the Mangold Pigmy beetle (*Atomaria linearis*) must not be confused with disease caused by Blackleg organisms (Plate XXV, figs. 1 and 2). Characteristic *Atomaria* damage is distinguishable from Blackleg by the small excavations that the beetle "bites out" from the seedling. This difference is well illustrated in Plate XXV, figs. 1 and 2. It is probable, however, that seedlings attacked by the Pigmy beetle may succumb to Blackleg, since spores may gain entrance to the tissues through the wounds that have been formed.

The infection of seed-clusters on stecklings or seed-plants occurs as the former are ripening. Such infection is preceded by the presence of spots and stripes, caused by *Phoma Betae*, on the branches and main stem; and, on these affected places, and afterwards on the seed-clusters, numerous black pycnidia are developed. Moreover, because of the large area exposed by the clusters owing to their irregular shape, and because of their absorbent nature, a favourable substratum is provided, not only for the initial establishment of *Phoma Betae*, but possibly for the collection of a heterogeneous assortment of organisms.

It seems probable that to some extent *Phoma Betae* is carried on from year to year by pycnidia on the "seed," and that infection of the seed-clusters follows, in natural sequence, various forms of plant infection, such as Blackleg on seedlings and the decay of older roots associated with *Phoma Betae*. The latter sometimes results from attack by the physiological disease, Dry Rot.

## II. SOME CONSIDERATIONS OF DAMAGE.

An accurate estimation of damage caused by infection of seed-clusters is difficult, but field experience has shown that it may be very severe. In the field the loss of "plant" is seldom noticed unless some patches or large portions of the field are barren, and as this frequently occurs, resort is often made to re-seeding or sowing to swedes. It is not only this obvious loss which should be considered, but the annual loss which is usually undetected and which is due to the failure of many of the sprouted seeds to show above ground. This loss is not brought to light by ordinary germination trials, because the exact count of the germinated "seeds" does not indicate the proportion of these which will have vigour enough to throw off the disease and appear above ground. Trials, discussed later, have shown how large is the proportion of seedlings which are prevented from showing above soil level and of which no indication is given in figures representing the percentage of germination. It was thought that if by the treatment of "seed" such losses caused by the presence of disease on the clusters could be avoided or be reduced to a stable level, a great step towards assuring an optimum "plant" establishment in the field would have been made. Neither too thin nor too thick a "plant" is desirable prior to singling; too thin a "plant" means a resulting crop below maximum, and too thick a "plant" extra labour in singling and also a lighter crop due to the decreased vigour of the young plants which are allowed to remain. The optimum establishment is therefore a vital factor determining the final yield. By repressing

Blackleg the number of stunted roots of low sugar content would also be reduced, and, in addition, the saving of "seed" which would be effected would be a desirable economy.

### III. PRELIMINARY TRIALS WITH DISINFECTANT "SEED" TREATMENTS.

#### (a) *Trials*, 1926.

In 1926 eight materials, namely, water,  $\frac{1}{2}$  per cent. and 1 per cent. mercuric chloride, 1 per cent. acetic acid, 1 per cent. and 2 per cent. carbolic acid, two organic compounds containing mercury, and a proprietary dust, were selected from a large number of recommended treatments to determine whether any of these increased the percentage of germination in the field or accelerated germination. Although it was found that none of the treatments accelerated germination in the field, an ortho-chlor-phenol mercury material<sup>1</sup>, and a 2 per cent. solution of carbolic acid<sup>2</sup>, both used as steeping solutions, increased the "plant" over that of untreated "seed."

#### (b) *Greenhouse trials*, 1928.

As the results of the above trials referred only to a "seed" sample, the percentage of infection of which was not determined, trials were carried out in early 1928, using "seed" of which the percentage infection was known.

Table I.

*Showing the mean percentage difference of the number of plants resulting from treatment and no treatment in greenhouse trials.*

Treatment	Mean (no. of plants)	Measure of significance of means	Mean percentage difference from untreated
Untreated (control) ... ..	55 $\pm$ 4.4	—	—
Mercury material* ... ..	76 $\pm$ 5.2	3.09	+ 38
Ortho-chlor-phenol mercury ... ..	66 $\pm$ 1.8	2.31	+ 20
Copper carbonate and mercuric chloride	44 $\pm$ 5.4	2.01	- 20

\* A proprietary material the active constituent of which is said to be cresolsodium mercuric cyanide, containing about 17 per cent. of mercury. It is applied at the strength of  $3\frac{1}{2}$  per cent., 1 quart being used to 12 $\frac{1}{2}$  lb. of "seed." The "seed" is then spread out on the floor or put in a mixing machine or agitator and mixed for three minutes. It is then spread out on the floor to dry partially before placing in the drill.

<sup>1</sup> A proprietary material the active constituent of which is said to be ortho-chlor-phenol mercury, containing about 17.4 per cent. of mercury. The strength at which it was used was 0.25 per cent., and in this diluted solution the "seed" was steeped for 2 hours.

<sup>2</sup> "Seed" was steeped for two hours.

For this purpose an ordinary commercial sample was obtained and this was examined by the Official Seed Testing Station, Cambridge. As the result of tests<sup>1</sup> it was found that 32.9 per cent. of the germinable seed-clusters produced growths showing disease lesions. Trials were then set out in a heated greenhouse in a large bed of unsterilised, fresh, garden soil. The means with their standard errors together with the measures of significance are given in Table I.

In Table I the significant increase given by the mercury material<sup>2</sup> should be noted.

In addition to these treatments four others were tried, namely, copper carbonate powder, a proprietary dusting powder, the latter powder mixed in equal proportions with copper carbonate, and the Dutch warm copper sulphate treatment<sup>3</sup>. The results obtained in these cases were not significant.

Estimations were made in all cases for Blackleg, but no significance could be attached to the figures obtained. The reason for this is manifest from the results of later experiments, in which it was found that the majority of the seedlings from untreated "seed" did not appear above ground; in these preliminary experiments this factor was not taken into consideration. It must be stated that in recording increase in "plant," only the last of five readings, taken at intervals between March 1st and July 3rd, has been taken as a basis for estimating the results. These readings, recorded for all treatments during the period mentioned, remained relatively constant. Since the minimum amount of water was given to the bed throughout the period of the experiment, the value of the mercury material for "seed" treatment under dry conditions for germination and establishment of the plants in the field seemed thus to be indicated.

Further greenhouse trials were carried out at Cambridge, and in each case the treated "seed" gave a significant increase of "plant" over the untreated.

#### (c) *Preliminary field trials, 1928.*

A parallel set of trials, using similar "seed" and similar treatments, was conducted at Oxford in the field, in duplicate plots. The "seed" was sown on April 3rd and counts were taken one month later. The means,  $\sigma$ , and odds as calculated by "Student's" method<sup>4</sup> together with the mean percentage of increase are given in Table II.

<sup>1</sup> Table VI.

<sup>2</sup> See footnote \*, p. 545.

<sup>3</sup> See *Attaak on sugar beet and mangold by Phoma Betae. Verslagen en Mededeelingen van den Plantenziektenkundigen Dienst te Wageningen*, No. 47.

<sup>4</sup> *Biometrika*, vi, 1-25, and xi, 414-17.

Table II.

*Showing actual mean increment in number of plants resulting from treatment together with  $\sigma$ , odds, and mean percentage increase.*

Treatment	Actual mean increment in no. of plants result- ing from treatment	$\sigma$	Odds	Mean percentage increase
Mercury material* ...	317	6.0	165	193
Proprietary powder ...	177	12.7	42	108
Ortho-chlor-phenol mercury	115	1.0	344	70
Copper sulphate (warm) ...	86	34.6	78	52

\* See footnote \*, p. 545.

Treatment with the mercury material resulted in a significant increase of "plant" of 193 per cent., and the proprietary dusting powder significantly increased the "plant" by 108 per cent. The latter substance thus gave results of significant value in the open in contrast to those obtained in the greenhouse.

In this trial, as in the former, warm copper sulphate was less efficient than the mercury material. For this reason, together with the fact that, for steeping at the exact stipulated temperature, the former treatment would necessitate special costly machinery and would also require apparatus for drying the "seed" afterwards, no further trials were made with it. The ortho-chlor-phenol mercury material also showed to better advantage in the field than in the greenhouse, but further trials with this were discontinued because the other material containing mercury proved superior in these trials.

Of the three remaining treatments, neither copper carbonate nor a substance containing a small percentage of mercuric chloride mixed with copper carbonate, nor a mixture of equal parts by weight of copper carbonate and the proprietary dusting powder, significantly influenced the "plant."

The "seed" used in the above trials was then replaced by an ordinary commercial sample obtained from the Eynsham Sugar Beet Factory, and was sown in the open in Oxford on May 14th after being treated with mercury material by the sprinkle method. The results obtained by "Student's" method from triplicate plots giving the respective means,  $\sigma$ , and odds together with the mean percentage of increase are given in Table III.

It is seen that by June 6th the "plant" had been increased by this treatment by 30 per cent.; by July 13th the significant increase over the untreated sample was 39 per cent. The percentage that showed typical Blackleg symptoms on lifting was 45 per cent. in the case of the treated

Table III.

*Showing actual mean increment in number of plants resulting from treatment together with  $\sigma$ , odds, and mean percentage increase.*

Treatment		Actual mean increment in no. of plants result- ing from treatment	$\sigma$	Odds	Mean percentage increase
Mercury material*					
1st count June 6th	...	93	5.5	52	30
2nd count July 13th	...	115	2.5	144	39

\* See footnote \*, p. 545.

and 5 per cent. in the case of the untreated sample. Although the latter results appear contradictory, it may safely be assumed, as a later trial indicates, that a large number of diseased seedlings remained below ground in the control, whereas the treatments allowed many of the attacked seedlings to appear which otherwise would have been prevented from doing so because of attack by the seed-borne fungi. It will also be seen from these trials that given favourable conditions for the development of the disease on the seed-clusters a high loss of "plant" is possible when an ordinary "seed" sample is sown.

(d) *Analysis of all seedlings above and below soil level.*

An analysis of all seedlings, above and below soil level, derived from untreated seed-clusters from the same sample of Kühn "seed" used earlier, is given in Table IV.

Table IV.

*Showing the number of seedlings with Blackleg that originated from untreated "seed."*

Total seed- clusters	Total not viable	Remaining below soil level		Appearing above soil level	
		Clean	Blackleg	Clean	Blackleg
723	39	397	566	62	57

The "seed" in this experiment was sown in large boxes in unsterilised soil in a greenhouse and analyses were made ten days after sowing. The greenhouse and soil conditions were ideal for fungal development in regard to moisture and heat. It can be seen that 59 per cent. of the seedlings which remained below ground showed symptoms of Blackleg.

(e) *Is the beneficial effect of wet treatment due to the influence of water or the chemical?*

As it was desired to prove whether the beneficial effects of wet treatments might be due entirely, or partly, to their wetting properties, the influence of water on the seed-clusters was investigated. Accordingly, "seed" of the variety Kühn, as used formerly, was wetted with water, at the same rate of application as that used in applying a solution of the mercury material; its influence was then compared with that produced by a solution of the mercury material applied at the normal rate. Both treatments were compared with controls which received no treatment and were sown in a dry state.

Each row in this trial contained 50 seed-clusters and each treatment comprised twenty rows, *i.e.* there were 1000 clusters altogether. They were set out in boxes of unsterilised soil in a greenhouse. It was arranged that each row of clusters receiving the water treatment was situated between one treated with the mercury material and another not receiving treatment. Twelve days after sowing, the number of plants which appeared in each row was counted. The results with their respective means, measures of significance, and standard errors together with the mean percentage increase are given in Table V.

Table V.

*Showing the mean number of plants obtained from "seed" untreated, treated with water, and treated with the mercury material respectively, together with percentage of increase by treatment.*

Treatment	Mean	Measure of significance	Percentage increase
Mercury material* ...	38.0 ± 2.80	5.16	90
Water ... ..	24.0 ± 3.01	1.09	20
No treatment ...	20.3 ± 2.08	—	—

\* See footnote \*, p. 545.

The increase of "plant" over that from untreated clusters, the significance, as may be seen, being very great, was 90 per cent. as the result of treatment with the mercury material, whereas water alone did not significantly affect germination, the measure of significance being 1.09. It seems conclusively shown that water was not the active constituent of the mercury material which had increased the "plant" in these trials.

(f) *Estimations in the laboratory of the percentage of diseased growths which arose from treated and untreated "seed."*

Prior to the laying out of the more extensive field trials, "seed" of the variety Kühn, taken from the same sample as that formerly used, and treated respectively with (1) carbolic acid,  $\frac{1}{2}$  per cent., steeped for half-an-hour, (2) the mercury material<sup>1</sup>,  $3\frac{1}{8}$  per cent., sprinkled on the "seed," (3) the proprietary dusting powder, and (4) untreated, was examined at the Official Seed Station for (a) the total diseased growths arising from clusters, (b) the number of normal growths, and (c) the number of clusters which did not germinate. Two hundred clusters from each treatment were pressed into moistened, sterilised sand, and a periodical examination was made during the germination trials. Ten seed-clusters were sown in each container of which there were twenty for each treatment. The results of the first, second and third tests conducted during the periods (1) Sept. 15th to Oct. 3rd, (2) Nov. 6th to Nov. 13th and (3) Nov. 15th to Nov. 23rd respectively, are given in Table VI.

Table VI.

*Showing the percentage of diseased growths which resulted from treated and untreated "seed" of the variety Kühn.*

Treatment	Test no.	Average percentage germination	Percent. of germinated clusters having diseased growths	Average percent. of clusters with diseased growths in tests nos. 2 and 3
Carbolic acid, $\frac{1}{2}$ per cent.	1*	85.5	94	32.1
	2†		16.5	
	3†		47.7	
Proprietary dusting powder	1*	91.5	73	8.5
	2†		7.5	
	3†		9.6	
Mercury material ...	1*	87.5	35	6.2
	2†		8.4	
	3†		4.1	
Untreated ...	1*	87.5	96	32.9
	2†		15.1	
	3†		50.8	

\* In test No. 1 conducted during the period Sept. 15th to Oct. 3rd only diseased growths were removed from the dishes, the remaining growths were left until the experiment terminated, so that a large proportion of the latter undoubtedly contracted infection through the diseased growths contaminating the sand.

† All germinated clusters were removed from the dishes on the 8th and 14th days.

It may be seen that the mercury material in tests No. 2 and No. 3 was the most effective in reducing the average percentage of germinated

<sup>1</sup> See footnote \*, p. 545.



clusters having diseased growths. It is also of interest to note the wide fluctuations in these percentages in the carbolic acid and untreated samples. The high percentage in test No. 1 is explicable since all growths were not removed at regular intervals as in tests Nos. 2 and 3. It is probable that in the second and third tests the temperatures were different, favouring the parasite or parasites in one case, and in the other case retarding them. It is seen that  $\frac{1}{2}$  per cent. carbolic acid was not effective in reducing the percentage of diseased growths. The proprietary dust on the other hand was quite effective in this regard.

(g) *Preliminary trials on the treatment of "seed" with sulphuric acid and comparisons with treatment with the mercury material.*

Sulphuric acid at strong concentrations has been used for the purpose of "decorticating" seed-clusters of sugar beet. This treatment effects a corrosive action on the exterior of the clusters, and as the result of carbonisation they become darker in colour and are considerably reduced in size. The treatment also serves to break up the clusters and thus allows the drill to distribute the "seed" more evenly and efficiently. The effect of the treatment as a disinfectant against *Phoma Betae*, and indirectly for the purpose of increasing "plant" establishment brought about entirely through its disinfecting action or partly through its action in stimulating the "seed," has received some attention during these investigations<sup>1</sup>.

In 1929, field trials were conducted at Oxford and Cambridge which were preceded by a preliminary test conducted in a greenhouse. The following treatments were employed: water, an organic compound containing mercury, 60 per cent. sulphuric acid, and the latter treatment followed by treatment of the dried "seed" with the organic compound just mentioned. The acid treatment consisted in steeping the "seed" in a 60 per cent. solution of sulphuric acid for a period of 45 minutes. This procedure was followed by submerging the "seed" in a large volume of water, so that thereby further action of the acid was prevented and, at the same time, disastrous results to the "seed" avoided which, in the event of a small volume of water being used, would occur owing to the evolution of heat. After washing in running water for several hours the "seed" was neutralised with a 1 per cent. solution of soda until litmus paper showed no change of colour. After drying the "seed" was ready for sowing. As the result of trials in the greenhouse it was

<sup>1</sup> Acknowledgment is made to Dr R. M. Woodman, Cambridge, for information concerning this treatment.

found that the mercury material mentioned allowed the greatest number of plants to be produced. The next treatment, in order of number of plants produced, was the combined acid and mercury treatment, and lastly came untreated "seed" and "seed" treated with water. Trials were then conducted outside, and an examination of the plots was made for the number of plants arising as the result of each of the treatments. The "seed" was sown on May 9th at Cambridge and on May 11th at Oxford in rows eight feet in length and six inches apart. Each treatment occupied one row and each contained 100 clusters sown by hand. Each treatment and the controls were replicated twelve times and were arranged in a randomized order in each series. The following table shows the mean number of plants resulting from each treatment.

Table VII.

*Showing the mean number of plants resulting from each treatment.*

Treatment	Mean no. of plants	
	<i>A</i> series	<i>B</i> series
Sulphuric acid ... ..	15	38
Mercury material* ... ..	37	59
Sulphuric acid and mercury material	31	50
Water ... ..	17	34
Untreated ... ..	21	31

S.E. of mean difference in Series *A* is 4.17 and in Series *B* 5.63.

S.E.  $\times 2.57$  in Series *A* is 11 and in Series *B* 14.

\* See footnote \*, p. 545.

Series *A* and *B* represent plots at Oxford and Cambridge respectively. The data have been analysed by the method utilising the average variance for any two plots<sup>1</sup>. The standard error of mean difference based on twelve pairs of plots is 4.17 in Series *A* and 5.63 in Series *B*. Any difference equal to or exceeding the figure obtained by multiplying the standard error by 2.57 will have a significance of 100 to 1. These figures are for Series *A* 11 and for Series *B* 14.

The difference between the mean number of plants in the water and untreated plots is less than the standard error in both Series *A* and in Series *B*, so that these treatments are not significantly different. It should also be noted that the mean differences in the case of plots treated with the mercury material, and those treated with sulphuric acid fol-

<sup>1</sup> *The Principles and Practice of Yield Trials*, F. L. Engledow and G. Udny Yule. Published by the Empire Cotton Growing Corporation, Millbank, London, S.W. 1, from vol. 11, Nos. 2 and 3, of *The Empire Cotton Growing Review*.

lowed by mercury material, are not significant in both series. The sulphuric acid and untreated plots do not significantly differ.

The mercury material used either as an additional treatment after sulphuric acid, or alone, was responsible for a greater number of plants than in the case of any of the other treatments, and the mean difference obtained was the only significant result. The increases resulting from the mercury material were 70 per cent. and 97 per cent. respectively in Series *A* and *B*.

#### IV. COMPARATIVE FIELD TRIALS WITH THREE "SEED" TREATMENTS.

Treated "seed" with untreated as controls was sown in field plots at the Cambridge University Farm and at the Farm Institute, Moulton, Northants. At Moulton the soil is light and sandy on ironstone formation, and the area which the plots covered had received similar treatment in cropping and manuring for many years. The "seed" was sown at the rate of 15 lb. per acre on May 3rd and 4th, 1928, using a five coulter drill; a strip of seven chains and composed of five drill-rows comprised each plot treatment. It was desired to find the effect of the dressings on:

- (a) "Plant" establishment.
- (b) Vigour of the seedlings as judged by dry and green weights, and the percentage of the plants attacked by Blackleg.
- (c) Yield, and the percentage of sugar.

- (a) *Field trials to estimate the "plant" establishment and percentage of Blackleg as the result of three "seed" treatments.*

The "seed" in the different plots showed little or no difference in their times of germination. The counts of the number of plants from ten one-yard strips were taken on June 8th immediately previous to singling; the strips were removed from the five drill-rows geometrically, in such a way that statistical analysis for the standard error was applicable to the results of the readings for each of the plots.

Table VIII.

*Showing for each treatment the mean number of plants with its standard error. The mean number of plants showing Blackleg is also included.*

	Mercury material	Proprietary dust	Carbolic acid $\frac{1}{2}$ per cent.	Untreated
Mean no. of plants ... ..	45 $\pm$ 5.46	19 $\pm$ 2.86	25 $\pm$ 2.12	20 $\pm$ 2.28
Mean no. of plants with Blackleg...	10	7	4	6

Table VIII gives the results from this method of examination, including in each case the mean number of plants with its respective standard error and the significance. The mean number of plants showing Blackleg symptoms is also included.

The measures of significance of the mean differences are: 4.14, 0.27, 1.16, and 2.28 respectively. The only significant increase of "plant" over the untreated was that due to the mercury material<sup>1</sup>, and this was responsible for the large increase of 125 per cent. As before, the differences between the means in the case of plants attacked by Blackleg were not significant.

At Cambridge, estimations showed that the mercury material increased the "plant" over untreated by 83 per cent. and similarly the proprietary dust by 70 per cent. and carbolic acid by 15 per cent. in one series of plots. The differences between the means of plants attacked by Blackleg were not significant as the plants above ground only were examined.

"Seed" from the same source was used in field plots at Moulton and Cambridge, namely, a commercial sample of which the proportion of germinable "seed" producing diseased seedlings was found to be 32.9 per cent.<sup>2</sup> All treatments, in these two field plots, were conducted by the same person, and each treatment was carried out in bulk so as to avoid differences due to the personal factor or those due to slight variations in method.

(b) *Vigour of the plants as judged by green and dry weights of seedlings derived from the treated and untreated samples.*

The average weight, both in the green condition and after air drying to constant weight, of seedlings from seed-clusters receiving each of the three treatments respectively, were compared with the corresponding weights of seedlings from untreated "seed." It was hoped that such comparisons would give some indication of the increased vigour of the plants due to "seed" treatment. The following table gives the average weights of seedlings in the trial plots at Cambridge. All the seedlings examined for "plant" establishment were included in obtaining the results noted in Table IX.

It may be seen that there is an average increase per seedling in both dry and green weight as the result of using each of the three treatments. The mean green weight and dry weight was increased by the treatments by 12 per cent. and 7.4 per cent. respectively, and thus field observations, which are indicative of increased vigour induced by treatment, were confirmed.

<sup>1</sup> See footnote \*, p. 545.

<sup>2</sup> See Table VI.

Table IX.

*Showing the average green weight and the average dry weight of seedlings from treated and untreated "seed" sown in the field.*

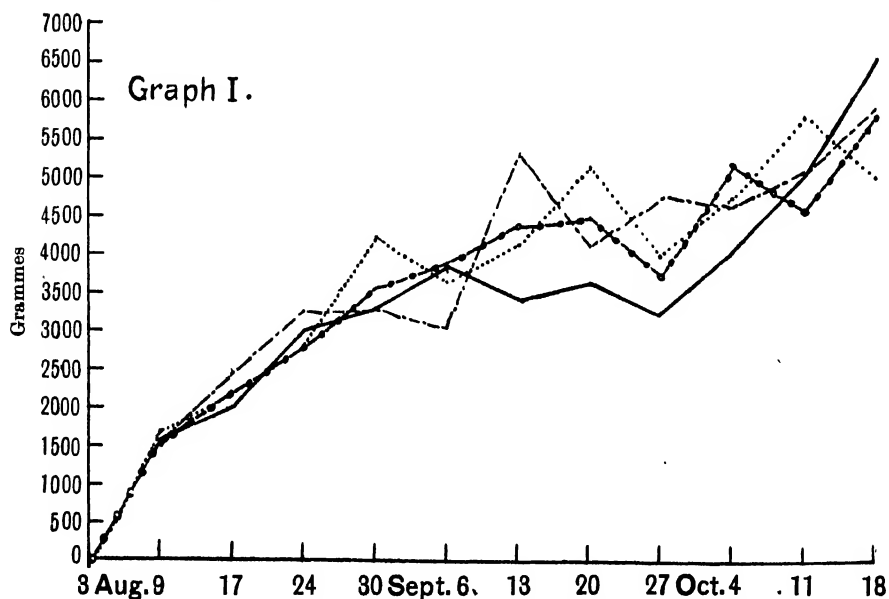
Treatment	Total seedlings	Average green weight in gm.	Average dry weight in gm.
Mercury material ...	486	0.58	0.0444
Proprietary dust...	610	0.58	0.0460
Carbolic acid, $\frac{1}{2}$ per cent.	648	0.51	0.0445
Untreated ...	370	0.50	0.0418

(c) *Effect of the treatments on yield and the percentage of sugar.*

The weights of the washed and "topped" roots, and the weights of the "tops," were taken at eleven weekly intervals in the four field plots at Moulton, the first weighing being made on August 9th. Thirteen beets were taken from each plot to obtain the average weight and, as

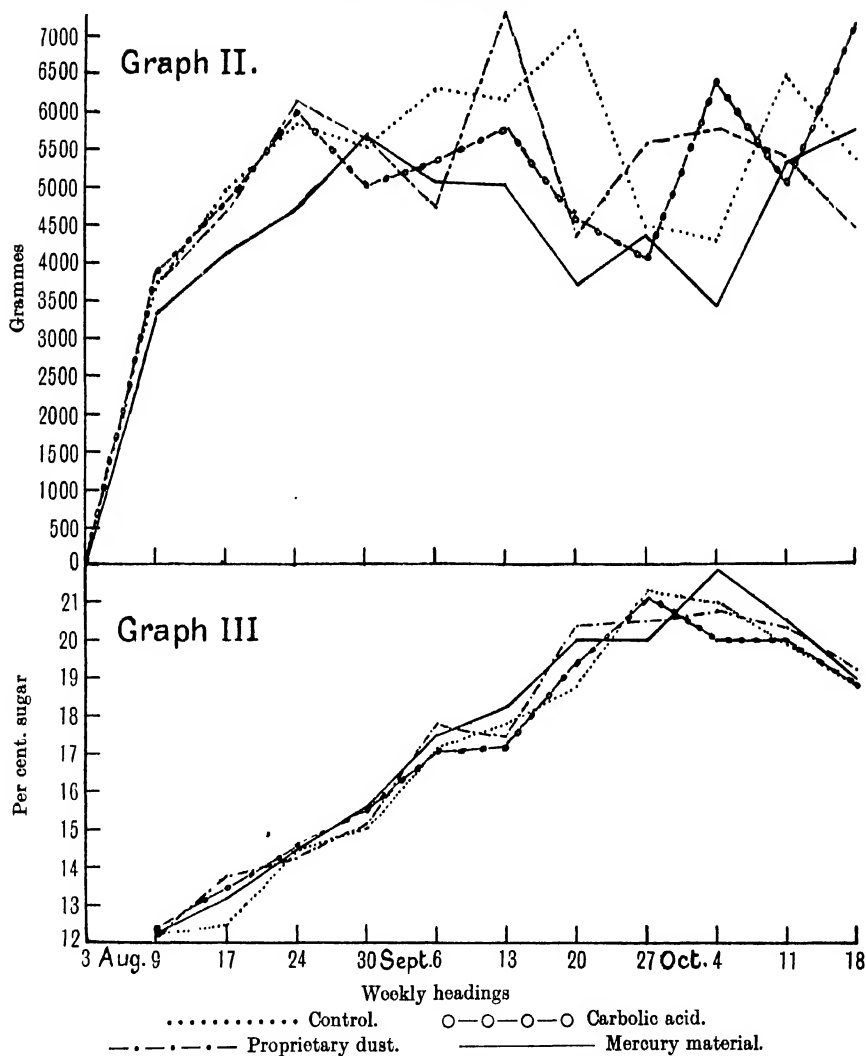
Table X.

*Showing in three graphs, the average weights of the roots after washing and "topping" and of "tops," and of the mean percentage of sugar from the four plots for eleven weekly readings taken between August 3rd and October 18th.*



Graph I. Showing average weight of washed and "topped" roots in grammes.

Table X (continued).



Graph II. Showing average weight of "tops" in grammes.

Graph III. Showing mean per cent. of sugar.

each beet was taken in such a way that the area representative of each plot was covered in obtaining the sample, an effort was made to obtain a representative average weight per beet for each of the treatments. The percentage of sugar was obtained at the same time and the results, presented in the form of graphs, are given in Table X.

In Graph I it may be seen that the weights of the washed and "topped" roots were about equal in the carbolic, proprietary dust and the untreated plots, and that these weights maintained a fairly steady upward trend. In the mercury plot the weight of the washed and "topped" roots did not significantly increase during September, but a steady upward trend is noted in October which finally slightly supersedes the weights obtained for the other three plots. As shown in Graph II, the fluctuations in the weights of the "tops" are less constant. In September a decisive drop in the weights of the "tops" in all the plots is noted and this is particularly reflected in the mercury plot in Graph I in the cessation of increase in weight during September of washed and "topped" roots. The weights of the "tops" in the final reading may be considered as about equal for all dressings if due consideration is given to the fluctuations which occurred earlier.

The percentage of sugar of the roots during this period is given for the four plots in Graph III. It may be noted that the percentage of sugar in all four plots was practically equal and maintained a steady upward trend until October. During the first week of October the percentage of sugar in each plot started at the same time on a somewhat downward trend but the end-point reached was practically identical. The decrease in the percentage of sugar was due to the rapid growth of new heart leaves during the first half of October. The highest sugar concentration occurred in the mercury material plot, namely 21.9 per cent. of sugar, in an analysis made on October 4. In summarising it may be said that there were only slight differences between roots in regard to weight of root, weight of "tops" and percentage of sugar as the result of the three treatments. The general reduction of the percentage of sugar in all treatments as the result of the growth of new heart leaves is a point of interest.

Table XI.

*Showing the final yield of washed and "topped" roots, "tops" and weight of sugar together with the estimated number of roots per acre for each plot.*

	Untreated	Mercury material	Proprietary dust	Carbolic acid
Weight of washed beet, in tons per acre	10.10	10.24	10.03	9.20
Weight of tops, in tons per acre	... 12.7	12.7	13.0	11.6
Weight of sugar, in tons per acre	... 1.93	1.88	1.93	1.79
Number of roots per acre	... 23,760	26,840	25,520	26,400

It may be seen that both the weight of the "tops" and the weight of washed and "topped" roots were decreased by approximately one ton

per acre through treatment with  $\frac{1}{2}$  per cent. carbolic acid, whereas neither the weights of the "tops" nor the weights of washed and "topped" roots were significantly affected by the mercury material or the proprietary dust. The number of beets per acre from "seed" treated with carbolic acid and from "seed" treated with the mercury material was slightly greater than the number produced in the case of the proprietary dust, and all three treatments produced a significant increase of mature roots over the number produced by untreated "seed." In the case of "seed" receiving the carbolic acid treatment a slight decrease in the weight of sugar per acre is noted. As may be seen there was no significant increase in weight of sugar per acre as the result of using either the mercury material or the proprietary dust. In summarising it may be said that  $\frac{1}{2}$  per cent. carbolic acid exerted an undesirable effect in reducing the weight per acre of "tops," "topped" roots and sugar. On the other hand the other two treatments did not exert undesirable effects.

V. EFFECT OF THE TREATMENTS ON COMMERCIAL "SEED" AS SHOWN BY THE RESULTS OF TRIALS IN WHICH TEN DIFFERENT VARIETIES OF "SEED" WERE TESTED.

The trials so far had been limited to three commercial samples of "seed" and the beneficial results from the use of the treatments had been observed only on the particular samples chosen. It was desired to find the effect of similar treatments on commercial "seed." Accordingly 26 samples of commercial "seed" were selected from different districts in different countries, and of these ten were taken at random to find the relative effects of the treatments. The dressings used were: (1) the mercury material, (2) a  $\frac{1}{2}$  per cent. carbolic acid, steeping process, and (3)  $3\frac{1}{2}$  per cent. carbolic acid, sprinkle method. Germination tests of the ten varieties were first carried out at the Official Seed Testing Station, Cambridge, with the results given in Table XI.

(a) *Effect on germination.*

It may be seen from these figures that the effect of the treatments on the final germination was very slight, but that "seed" treated with mercury material was a little longer in germinating than other samples under the conditions of this experiment. This delayed germination has not been noted in any of the field trials.



Table XII.

*Showing the percentage of germination of ten different sugar beet varieties treated and untreated.*

Variety	Test no.	Mercury material % germ *		Carbolic† ½ % % germ *		Carbolic ¾ % % germ *		Control % germ		
		6 days	Final	6 days	Final	6 days	Final	6 days	Final	
Sharpe's WZ	...	1	57	72	68	72	70	75	69	71
		2	72	75	—	—	—	—	—	—
Debrovice	...	1	60	73	64	69	58	72	73	76
		2	26	55	—	—	—	—	—	—
Hilleshog	...	1	65	82	70	77	83	88	72	80
Braune	...	1	75	85	90	93	91	94	90	93
Dippe	...	1	81	89	82	84	83	86	76	78
Kleinwanzleben	...	1	70	75	74	77	69	75	70	73
		2	44	69	—	—	—	—	—	—
Kühn	...	1	74	88	81	86	84	88	84	88
Schreiber	...	1	55	81	80	81	77	82	72	75
Rakovsky	...	1	71	83	84	86	87	89	86	88
Zapotil	...	1	49	69	72	79	69	78	71	78

\* I.e. percentage of clusters each giving at least one seedling.

† One quart to 12½ lb. of "seed."

(b) *Effect on "plant" establishment.*

"Seed" from the same treated and untreated sample lots was sown in Oxford in a well prepared, even, friable seed-bed on July 6th and 7th, 1928. Of each of the three differently treated samples of "seed" of the ten chosen varieties two hundred clusters were sown by hand in rows

Table XIII.

*Showing the mean difference from untreated,  $\sigma$ , the odds and the mean percentage difference.*

Variety	Treatment	Mean difference	$\sigma$	Odds	Mean per cent. difference from untreated
Dippe	Mercury material*	106	54.8	103	+48
Debrovice	Mercury material*	61	21.2	434	+30
	Carbolic ¾	50	12.7	1427	+27
Hilleshog	Mercury material*	88	49.1	85	+46
Zapotil	Carbolic ½	72	19.2	1101	+36
	Mercury material*	23	20.6	20	+12
Kleinwanzleben	Carbolic ¾	44	27.5	60	-24
Braune	Carbolic ¾	63	54.1	23	-19
	Carbolic ½	69	37.7	824	-21
Sharpe's WZ	Carbolic ¾	19	4.6	1665	-12

\* See footnote \*, p. 545.

eight feet in length replicated five times. All the rows comprising the ten varieties were laid out in randomised fashion, but each row had an adjoining row sown with untreated "seed" from the same variety sample. "Student's" method was used to calculate the significance of the difference of means. For the sake of brevity those results not significant are not included in Table XIII.

These trials showed that four of the varieties, namely Dippe, Debrovice, Hilleshog and Zapotil, gave a positive response of significant value to treatment with the mercury material as shown by an increase of "plant" ranging from 12 per cent. to 48 per cent. The remaining six varieties, namely Kühn, Schreiber, Rakovsky, Sharpe's WZ, Braune and Kleinwanzleben were not significantly affected adversely or advantageously by this treatment.

Carbolic acid,  $3\frac{1}{2}$  per cent., significantly increased the "plant" of Debrovice by 27 per cent. but significantly decreased the number of seedlings in the three varieties Kleinwanzleben, Braune and Sharpe's WZ by 24, 19 and 12 per cent. respectively. The remaining varieties were not significantly influenced by the  $3\frac{1}{2}$  per cent. carbolic acid treatment.

One variety, Zapotil, gave a significant increase of "plant" of 36 per cent. after being treated with  $\frac{1}{2}$  per cent. carbolic acid, but the same treatment significantly decreased the "plant" of Braune by 21 per cent. The remaining eight varieties treated with  $\frac{1}{2}$  per cent. carbolic acid were not significantly affected.

A long period of drought followed sowing, and this was undoubtedly responsible for reducing the obvious effects of treatment on establishment of "plant," but, even so, one of the treatments, namely that using the mercury material, was distinctly beneficial to four varieties and did not disadvantageously affect the remainder. Steeping "seed" in  $\frac{1}{2}$  per cent. carbolic acid, which is a very old specific treatment on the Continent for sugar beet, and also a modified form of this using the acid at a strength of  $3\frac{1}{2}$  per cent. for sprinkling on the "seed," proved of little value under these conditions; the latter significantly decreased the "plant" of three varieties, and neither of the treatments was responsible for a general improvement of "plant."

At Cambridge similar plots were laid out and field observations indicated that no obviously adverse effect had followed the treatment of "seed."

- (c) *The relationship of the percentage of germination of the "seed," and percentage of germinated seed-clusters producing diseased growths to the mean percentage of increase in the number of plants in response to treatment.*

The ten varieties used in these trials were then submitted to the Official Seed Testing Station to be examined for diseased growths. The results are given in Table XIV.

Table XIV.

*Showing the percentages of germination of diseased growths, and of increase in the number of plants as the result of treatment with the mercury material.*

Variety	Per cent. of germination	Per cent. of germinated clusters with diseased growths	Percentage increase of "plant" by treatment*
Braune ...	94.5	95.0	—
Rakovsky ...	91.0	95.0	—
Dippe ...	88.5	90.5	48
Schreiber ...	83.0	94.6	—
Hilleshog ...	80.5	91.3	46
Debrovice ...	83.5	84.5	30
Kleinwanzleben ...	95.5	90.7	—
Kühn ...	88.0	77.4	—
Zapotil ...	84.5	75.0	12
Sharpe's WZ ...	78.0	57.0	—

\* Table XIII.

Each of the results in the first two columns was obtained after the examination of 200 clusters which had been germinated in sterilised sand. As in previous trials<sup>1</sup> all growths were removed as they appeared. Firstly, it may be noted that there is no correlation existing between the percentage of germinated clusters which produced infected growths and the percentage of germination. Secondly, this examination had shown how high is the percentage of infected growths which develop from "seed" of the majority of commercial varieties. Concerning the four varieties influenced by the mercury treatment in a former trial<sup>2</sup> there is a direct correlation between the percentages of clusters producing diseased growths and the percentages of increase of "plant" by this treatment.

<sup>1</sup> See Table VI and following paragraph.

<sup>2</sup> Table XIII.

It appears, then, that the greater the intensity of the disease in a sample the greater is the beneficial action of the treatment. An inference which may be drawn from this fact is that, although the percentage of infected growths as obtained from these trials does not necessarily correspond with that proportion of seedlings seriously handicapped or killed by disease, this percentage does serve to indicate the relative intensity of attack controllable by treatment. It is obvious, then, taking two extremes encountered in these trials, that, if so large a proportion as 95 per cent. of the germinable clusters of a certain variety produced diseased growths, the intensity or pathogenicity of controllable disease is greater than it is in the case of a variety in which 57 per cent. of the "seed" produced diseased growths. This aspect of attack should be considered in interpreting these results. For example, the relatively small increase of "plant" of 12 per cent. in the case of Zapotil "seed," infected to the extent of 75 per cent., may be explained by the hypothesis that the lethal action of the disease in this case would be much less prominent than it would be in the case of Dippe "seed" infected to the extent of 90.5 per cent. This was actually shown to be the case as it can be seen that the response of the latter to treatment was much more marked as shown by a significant increase of "plant" which was 48 per cent. greater than the "plant" derived from untreated "seed."

(d) *Preliminary observations relating to commercially supplied "seed" treated with the mercury material.*

In co-operation with a number of sugar beet factories endeavour was made to make as many observations as possible in the field where treated<sup>1</sup> "seed" supplied commercially was being sown. Such endeavours in nearly every case were unfruitful. It was found that no observations were of value unless the lay-out of plots and supervision of all details concerning the soil, sowing of the "seed," etc., were in the hands of an experienced officer. It was found that the number of factors, any of which could bias the results of observations, was very great.

On the Cambridge University Farm an effort was made to compare the respective number of plants resulting from untreated "seed" and treated "seed" purchased as such. The "seed" was sown at the same rate per acre by means of a drill. Sampling was accomplished by selecting ten representative strips of the drill-row 50 feet in length, in such a way as to cover representatively the area occupied by each treatment. The

<sup>1</sup> Treated with the mercury material, cf. footnote \*, p. 545: method of treatment not known.

mean number of plants in each case, together with the measure of significance and standard errors, are shown in Table XV.

Table XV.

*Showing the mean number of plants resulting from commercial "seed" untreated and treated. The percentage of Blackleg is also given.*

Treatment		Mean number of plants	Per cent. of Blackleg	Average per cent. increase by treatment
Untreated	...	70 $\pm$ 6.9	8.7	—
Mercury material	...	99 $\pm$ 14.4	4.4	41.5

The measure of significance of the difference of the means is 1.85, thus being barely significant. The average increase of 41.5 per cent. should be noted. The average dry weight per seedling was found to be 0.0488 in the case of untreated "seed" and 0.0530 in the case of treated "seed," *i.e.* an increase of 8.6 per cent. In a former test it will be noted<sup>1</sup> that the average increase in dry weight per seedling as the result of the three treatments was 7.4 per cent. In the table to which reference is made it will also be noted that the increase by the mercury treatment was 6.2 per cent. per seedling.

It will be necessary to make more extended tests of treated "seed" offered to growers. It is suggested that "seed" is not always as efficiently disinfected in bulk as in the carefully supervised treatment of relatively small quantities. For this reason "seed" treated in the course of these investigations does not compare with treated "seed" from suppliers. It is also suggested that the results obtained from such "seed" would be incomparable for another reason, namely, moisture content. "Seed" treated under personal supervision and used in these trials was sown when relatively wet. The standard to be aimed at was unhindered delivery by the drill and the avoidance of inconvenient clogging. Data<sup>2</sup> are to hand which show that in many cases only partial disinfection was attained as shown by the examination of treated "seed" offered to growers. The results of the examination for the presence of *Phoma Betae* together with relevant data are shown in Table XVI.

In addition to the samples, to which reference is made in the following table, four others representing three well-known varieties were examined and found to be infected by *Phoma Betae* to a negligible extent, so that re-treatment was not undertaken. To obtain the percentages in the last

<sup>1</sup> Table IX and preceding paragraph.

Published by the consent of the English Beet Sugar Corporation Limited.

Table XVI.

*Showing the percentage germination and infection of five samples of commercial treated "seed"; also the percentage increase in the number of growths and decrease in the number of diseased growths after re-treatment\*.*

Variety	Per cent. germination	Per cent. infection	Per cent. increase in the number of growths after re-treatment	Per cent. decrease in the number of diseased growths after re-treatment
A†	86	50	21	95
B	87	25	8	82
C	84	36	46	75
D	—	34	24	92
E	90	51	24	67

\* A  $\frac{1}{2}$  per cent. solution of the mercury material was used in which the "seed" was steeped for two hours.

† A-E represent samples of four common varieties.

two columns 100 clusters were allowed to germinate and the total number of growths which appeared was compared with that obtained from "seed" as supplied in its original treated state. It can be seen that there is a great variation in the percentage of infection of treated "seed" as received from suppliers. The efficacy of re-treatment is shown.

## VI. DISCUSSION.

It is highly desirable that the "plant" should not fail in the field when circumstances favour seedling diseases, as they do in certain years, and it cannot be denied that it is a wise precaution to insure against such losses which, although they may be of little consequence one season, might be of vital importance in another. Furthermore, the small cost and little trouble which is required in the application of one of the "seed" treatments are of small account when compared to the advantages which may be derived. It cannot be said that such treatment of "seed" would result in an increased yield or an increased percentage of sugar in any one season, but there is always the possibility that under conditions favourable to seedling diseases the yield would be increased.

It seems probable that the optimum "plant" would be established in the field if it were possible to judge more accurately the amount of "seed" to be sown per acre; this, assuredly, would be made more simple by using either disease-free "seed" or treated "seed," as then much would be accomplished towards assuring an even "plant" by lessening the effects of that variable factor the infection of "seed." The optimum

"plant" as already mentioned, is of considerable importance, for it must be pointed out that too thick a "plant" as well as too thin a "plant" must both exert an adverse effect on yield. The real although less obvious effect of too thick a "plant" is to reduce the vigour of the plants left after singling by previously crowding together the seedlings in the row.

In conclusion, a reference to the views expressed in a recent paper by Engledow and Maher is appropriate in pointing out the importance of obtaining a full and even plant population. Their conclusions are as follows: "It is in sugar beet, of all our field crops, that a full plant is to be expected. In common with other root crops it is singled, only the most suitable fields are selected for it, preparatory cultivations and making of the seed bed are done with great care, and it is a spring crop. Further, it will be shown in later passages that the Continental insistence on full even plant as a guarantee of maximum yield is strictly applicable to our own conditions<sup>1</sup>." And again, "But the new crop is still on trial. It has to bear the grievous burden of a disappearing subsidy. Greater yield per acre is its only hope, and this, it is believed, is to be realised mainly through a full and even plant<sup>2</sup>."

## VII. SUMMARY.

1. The organisms responsible for Blackleg in sugar beet seedlings have been referred to and the damage that they do considered.

2. It has been shown that treatments used to disinfect the "seed" may be harmful, they may exert no influence, or they may be of considerable value.

3. The present trials have shown the beneficent effects of an organic compound containing mercury. This material increased the "plant" in the majority of cases, and this increase varied between 12 per cent. under some conditions and in increasing amounts up to 193 per cent. under more favourable conditions.

4. An increase in the vigour of young plants was noted occasionally. Quantitative tests showed an increase in both the green and dry weight per seedling as the result of treatment. The beneficial action of treatment is not due to the influence of water.

5. The organic compound containing mercury was the most effective in reducing diseased growths during laboratory tests; the proprietary

<sup>1</sup> Engledow, F. L. and Maher, C. A. "Yield and plant population in sugar beet." *Journ. Agri. Sci.* XVIII, pt 4 (Oct. 1928), 578.

<sup>2</sup> *Ibid.* p. 590.

dust was nearly as efficient. Steeping in a  $\frac{1}{2}$  per cent. solution of carbolic for half-an-hour was ineffective.

6. Treatment of the clusters with 60 per cent. sulphuric acid did not appreciably improve "plant" establishment, but this treatment followed by the mercury treatment increased the number of plants considerably.

7. Field trials conducted for one season on a field scale have not shown an increase in the percentage of sugar as a result of the treatment of "seed," but a slight increase in yield and in the total number of roots per acre was noted as the result of treatment with the organic compound containing mercury. The proprietary dust was not noted as being responsible for reducing the "plant" in the field. Its beneficent action was not as uniformly apparent as that of the mercury material.

8. Carbolic acid at a strength of  $\frac{1}{2}$  per cent. was definitely injurious when used for steeping purposes. Furthermore, trials show no benefit from a modified form of this treatment in which a  $3\frac{1}{2}$  per cent. solution is sprinkled on the "seed." Other materials tested were found to be of doubtful value.

9. Certain samples of commercial "seed" as supplied treated with a mercury material were found to be infected to a considerable extent by *Phoma Betae*. Such "seed" it was found could be efficiently re-disinfected by careful re-treatment with marked beneficial results.

In conclusion it is desired to thank those who gave assistance. Among these are Mr Alfred Eastham, Director of the Official Seed Testing Station, Cambridge, Dr Van Poeteren, Wageningen, Mr G. R. Clarke, Advisory Chemist, and Dr N. Cunliffe, Advisory Entomologist both of Oxford, Mr W. A. Stewart and Mr W. R. Seward, Dr Pethybridge and the officials of many beet sugar factories who lent a helping hand.

#### EXPLANATION OF PLATE XXV

Fig. 1. Blackleg damage.

Fig. 2. Pigmy beetle damage.

(Received April 7th, 1929.)





FIG. 2.



FIG. 1.



## STUDIES IN BACTERIOSIS. XVI

THE AGGLUTINATING AND PLASMOLYTIC ACTION OF THE  
SAP OF THE POTATO ON VARIOUS PARASITIC AND SAPRO-  
PHYTIC SPECIES OF BACTERIA

BY EMILY M. BERRIDGE, D.Sc.

*(Imperial College of Science and Technology, London.)*

(With 2 Text-figures.)

IN recent work on plant immunity the tendency has been to emphasise the mechanical defence against invading organisms which a plant possesses, due to its cell walls and cuticle, and also to its power to cut off the diseased portion of its tissues by rapid cork or gum formation without serious detriment to the plant as a whole. But it is evident that the susceptibility of a plant to attack, or its power of resistance, must often depend on delicate reactions between the cells of the host and the attacking organism which are difficult to detect. In the animal body the flocculation of bacteria by agglutinins in the blood is a delicate reaction of this nature, indicating some conditions unfavourable to the invader. The evidence for the presence of agglutinins in plant juices is on the whole unsatisfactory. In several cases specific agglutinins, which appear after invasion by bacteria, have been detected, but the evidence for permanent natural agglutinins is extremely scanty. The specific agglutinins are usually localised in the tissues near the infected area. Schiff-Giorgini<sup>(14)</sup> showed that a water extract of the cortical tissues of *Olea europaea* adjacent to a tumour caused by *B. oleae* (*B. savastanoi*, E.F.S.) possessed agglutinating and bactericidal properties, which were considerably greater in amount than those possessed by a water extract of healthy tissues. Kritchewsky<sup>(7)</sup> demonstrated the presence of precipitins and agglutinins in the juice of *Cotyledon Scheideckeri* after inoculation with *B. typhi* and *B. cholerae*, and Korinek<sup>(6)</sup> obtained marked agglutination with the juice of *Beta vulgaris* infected by *B. tumefaciens*, but regarded it as due to absorption on crystals and coagulated colloids of the sap, and not true agglutination. Wagner<sup>(15)</sup>, too, observed the agglutination of *B. vulgatus*, *B. putidum* and *B. astero-*

*sporus* by the juice of the potato into which they had been inoculated. Vigliano is quoted by Korinek(6) as having found natural agglutinins in the sap of many plants, but says that Carbone denies the formation of any agglutinins in the sap of the potato, even after infection with either parasitic or non-parasitic species of bacteria.

It will be evident from the experimental work recorded in the following pages that these conflicting results are probably in part due to the fact that more delicate methods than those usually employed are needed to detect agglutination in plant juice, and also to the fact that the agglutinating and bactericidal reactions between the organism and plant vary with each species investigated.

In cases where bacteria inoculated into a certain host plant fail to produce parasitism of that host, and are agglutinated, this agglutination cannot be due to the H-ion concentration of the sap alone, since it has been shown by previous work (Berridge(2)) that H-ion by itself acts injuriously on various parasitic and saprophytic bacteria of common occurrence only at concentrations above *pH* 5.0-4.4; and Rea and Small(13), Clevenger(4) and others have shown that concentrations less acid than this prevail as a rule in adult plants. It seemed possible, however, that the agglutinating action might be exerted by acid combined with buffer substances in the sap, or by traces of trivalent elements such as iron or manganese present in it, since the salts of such metals have been shown by Eisenberg(5), Bechhold(1) and others to have an agglutinating action on bacteria even in extremely dilute solutions.

Phosphates, which are important buffer constituents of cell sap, are precipitated from plant juices when strong alcohol is added in excess, together with traces of salts of calcium, magnesium, iron and manganese, if present. The agglutinating effect on various types of bacteria of such a precipitate was therefore investigated as it might reasonably be inferred that any action possessed by such a solution would also be exercised by the cell sap itself.

#### PREPARATION OF THE PRECIPITATE AND METHODS OF EXPERIMENT.

Peeled potatoes, usually of the variety "King Edward," were grated, and the mush packed as quickly as possible into a Büchner funnel over a thin layer of asbestos; the juice was drawn through by means of a filter pump, and immediately poured into five times its volume of 95 per cent. alcohol. A bulky precipitate appeared, this was allowed to settle overnight, the alcohol was then decanted off and the sediment dried at about 52° C. The precipitate was prepared in small quantities and

kept in a desiccator, and the solutions made up as wanted, since the latter tended to change on keeping. When required for tests the precipitate from about 4 c.c. of juice was dissolved in distilled water, being kept at about 52° C. for ten minutes; the reaction of this solution was 5.8-6.0, *i.e.* roughly that of the potato juice itself. The water had previously been autoclaved in order to drive off the CO<sub>2</sub> and reduce the pH to 7.0, and to eliminate contaminating organisms as far as possible from the tests. The volume of water used was half that of the juice from which the precipitate was obtained, so that on adding an equal volume of a bacterial emulsion, the total dilution of the active substance was, as far as could be secured, the same as the dilution in the juice itself. A large proportion of the sediment did not dissolve, and was filtered off before the solution was used. The bacterial emulsion was prepared from cultures on bouillon agar grown for 24-48 hours at 25° C. The growth was removed and emulsified with a platinum loop and not washed off, in order to avoid the introduction of soluble salts from the medium.

This solution of the alcoholic precipitate from potato was found to possess an agglutinating action on certain types of bacteria, but not on others. This agglutinating power was not destroyed by boiling the solution for 30 minutes, nor wholly lost after standing exposed to the air for 3 or 4 days.

The agglutinated clumps of bacteria did not as a rule sink to the bottom of the tube, but formed a pellicle at the surface of the liquid, or remained suspended in it. In many cases they were microscopic in size, and where agglutination was followed by plasmolysis, the bacteria were reduced to granules. Therefore films, usually stained with carbol fuchsin, were always made, in order to determine whether agglutination or plasmolysis had taken place; these were examined with a  $\frac{1}{15}$ th inch oil immersion lens, to make sure that chemical precipitates in the liquid were not mistaken for bacterial clumps. The course of the reaction was followed by making films  $\frac{1}{2}$ , 2 and 4 hours after the beginning of the test. The films made after  $\frac{1}{2}$  hour, or earlier in cases where rapid plasmolysis rendered this necessary, served as controls, indicating the density of the emulsion and freedom from clumps. The 4-hour films furnished the results summarised in Table I, while the 2-hour films were useful in checking these results, especially in cases where plasmolysis was occurring. The comparative number and size of the clumps could be observed in the films, but many free bacteria were always present, since in the process of filming the clumps were partly broken up.

Fourteen different species of bacteria were tested, representing a variety of types:

<i>B. carotovorus</i>	...	...	Soft rot of carrot, turnip, etc.
<i>B. solanisaprus</i>	...	...	Rot of potato.
<i>B. phytophthorus</i>	...	...	Blackleg of potato.
<i>B. delphinii</i>	...	...	Leaf-spot of delphinium.
<i>B. malvacearum</i>	...	...	Leaf-spot of cotton.
<i>B. marginale</i>	...	...	Blight of lettuce.
<i>B. pyocyaneus</i>	...	...	Occasionally parasitic on plants and animals.
<i>B. fluorescens liquefaciens...</i>			Non-parasitic fluorescent type.
<i>B. fluorescens non-liquefaciens</i>			" "
<i>B. tumefaciens</i>	...	...	Causing galls on plants.
<i>B. coli</i>	...	...	Non-parasitic on plants.
<i>B. vulgare</i>	...	...	" "
<i>B. mycoides</i>	...	...	Sporing saprophytic type.
<i>B. dendroides</i>	...	...	" "

#### AGGLUTINATION TESTS.

The 14 species above enumerated were subjected to three series of agglutination tests, using (1) the solution of the potato precipitate at pH 6.0, the natural reaction of the expressed juice; (2) the fresh juice immediately after filtration; and (3) the solution of the potato precipitate with its H-ion concentration varied by addition of acid or alkali.

(1) The first series of tests with the precipitate solution at pH 6.0 showed that it was precisely those types which can parasitise potato, *i.e.* *B. solanisaprus* and *B. phytophthorus*, which were least affected by the substances thrown down by alcohol from the sap. *B. carotovorus*, *B. delphinii* and *B. vulgare* were also little affected, but *B. coli*, *B. tumefaciens*, *B. malvacearum*, and *B. marginale* were distinctly agglutinated in small clumps, while the two species of *B. fluorescens*, *B. pyocyaneus*, and the sporing organisms were strongly flocculated. Broadly speaking, the parasitic forms were not influenced by the substances in the solution, or only to a comparatively slight degree, while the non-parasitic forms were strongly agglutinated.

(2) The second series of tests, in which the fresh juice was used immediately after filtration, proved that the agglutinating substances of the precipitate were active in the untreated juice, for the degree of agglutination of the various species was about the same as in the previous tests. The juice, however, possessed a much greater plasmolytic power than the precipitate solution. The degree of plasmolysis after 4 hours varied with the species tested, but did not correspond in any way with

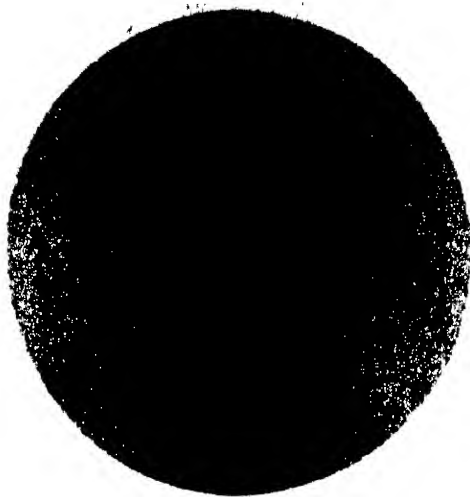


Fig. 1 Film from an emulsion of *B. solanisaprus* showing no agglutination by the potato precipitate solution after four hours

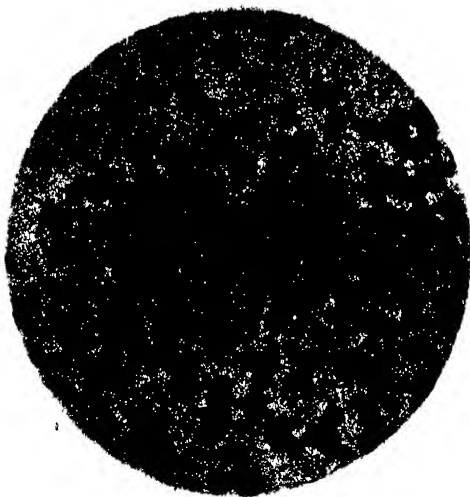


Fig 2 Film from an emulsion of *B. fluorescens liquefaciens* showing almost complete agglutination of the bacteria by the potato precipitate solution at the end of four hours

the degree of agglutination. The two potato parasites and *B. carotovorus*, which has been known to attack potatoes occasionally (Lacey (8)), remained almost unagglutinated and also unplasmolysed, but among the non-parasitic species, while the strongly agglutinated *B. fluorescens* and *B. pyocyaneus* resisted plasmolysis, in the *B. coli* and *B. vulgare* bacilli the cell contents were mostly reduced to granules within  $\frac{1}{4}$  hour. The emulsions of sporing forms very quickly showed nothing but spores. Among the plant parasites, too, while *B. marginale* showed little plasmolysis of the agglutinated clumps after 4 hours, *B. delphinii* and *B. tumefaciens* were almost entirely plasmolysed. Agglutination therefore appears to be due to substances which can be precipitated from the juice by 95 per cent. alcohol, while this plasmolytic agent is evidently not precipitated to any great extent. The latter also differs from the agglutinating agent by being gradually lost or destroyed when the juice is exposed to the air; juice which had been left standing in the air for 24 hours and then filtered, exerted little plasmolysing effect on *B. vulgare* during 4 hours, and *B. coli* was only partially plasmolysed at the end of 2 hours; also after being boiled and kept for some time it showed practically no such power. This plasmolytic power of the juice must therefore be due to something in the fresh sap which is either oxidisable or volatile.

(3) In order to test whether H-ion concentration had any influence on these reactions, the potato precipitate solution was acidified, usually with weak acetic acid, or made alkaline with dilute ammonia or caustic soda. The pH values were determined colorimetrically by comparison with Clark's colour chart (3) in a parallel series of agglutination tubes directly after the test was set up, and frequently again at the end of 4 hours' incubation. The results with ammoniated solutions had often to be discarded owing to considerable change in reaction, and *B. coli*, in particular, showed a great tendency to neutralise an alkaline environment even in the space of 4 hours.

This third series of tests showed that in the presence of the particular constituents forming the precipitate there is for each species a certain H-ion concentration at which it suffers neither agglutination nor plasmolysis. For *B. solaniasaprus* and *B. phytophthorus*, species which are pathogenic to potato, this value is pH 6.2, which is approximately the value of the H-ion concentration of the juice of the potato itself. With methyl red the reaction of the sap appeared to be pH 5.8-6.0; Wagner (15) determined it as 5.8 with lackmosol, while Youden and Denny (16), using slices of tissue immersed in various indicators, give the value as 6.1. This last value is probably nearest to the true one for the unoxidised



sap, for there is a slight but distinct rise of acidity on breaking up the tissue and thus exposing it to the air.

The complete list of pH values at which the 14 species tested are neither agglutinated nor plasmolysed by the potato precipitate is given in the first column of Table I.

Table I.

	Agglutination tests with potato precipitate		Cultural tests in Uschinsky solution	
	Bacteria unaffected pH	Bacteria plasmolysed pH	No growth pH	Growth pH
<i>B. fluorescens liquefaciens</i> ...	5.0	4.4	4.6	4.8
<i>B. fluorescens non-liquefaciens</i> ...	5.0	4.4	4.6	4.8
<i>B. pyocyaneus</i> ...	5.0	4.4	4.6	4.8
<i>B. marginale</i> ...	5.2	4.4	4.6	4.8 aggl.
<i>B. tumefaciens</i> ...	5.6	4.6	4.6	4.8
<i>B. delphinii</i> ...	6.0-6.4	5.2	5.2	5.5 slight
<i>B. malvacearum</i> ...	6.0-6.2	5.2	5.2	5.5
<i>B. solanisaprus</i> ...	6.2	4.6	4.6	5.2 slight
<i>B. phytophthorus</i> ...	6.2	5.2	5.2	5.5 slight
<i>B. carotovorus</i> ...	6.4-6.8	5.2	5.5	5.8
<i>B. vulgare</i> ...	7.2	5.8	6.4	6.9
<i>B. coli</i> ...	7.4	5.8	5.8	6.4 slight
<i>B. dendroides</i> ...	7.6	6.0	6.4	6.9 slight
<i>B. mycoides</i> ...	7.6-7.8	6.8	6.9	7.4 aggl.

For the plant parasites these points of non-agglutination all lie between pH 5.0 and 7.0; it is doubtful whether the *B. fluorescens* species ever become parasitic, but the closely related *B. pyocyaneus* can attack lettuce (Mehta and Berridge (11)). In the cases of *B. carotovorus* and *B. delphinii*, the points of non-agglutination were not well defined, but, for the former, which has been known to attack potato occasionally (Lacey (8)), it is slightly less acid than that of the true potato parasites.

For *B. coli*, as might be expected, a slight degree of alkalinity is favourable, while for *B. vulgare* the pH value lies near the neutral point. The two sporing organisms are hardly ever obtained in an emulsion of free bacteria, since they agglutinate spontaneously, but they grow most vigorously at about pH 7.6-7.8.

Each of these pH values may be called an "optimum" pH value for the species, since, judging by the case of the two potato parasites, it appears to be the degree of acidity most favourable for the attack of the species on the plant. It represents, however, the point at which the injurious effect of the sap on the invading organism is a minimum, rather than any true optimum condition of the bacterium.

On either side of this "optimum"  $pH$  concentration for any one species, change in either an acid or alkaline direction brings about agglutination. This increases with increasing change of  $pH$ , till plasmolysis sets in, due in this case to high  $H$ -ion or  $OH$ -ion concentration. This plasmolytic effect, which is not lost on exposure to the air, seems to be independent of that brought about by the fresh juice. Taking *B. coli* as an example, the "optimum"  $pH$  is 7.4, as determined by three independent tests. At  $pH$  6.6 the emulsion is agglutinated after 4 hours, at 5.8 this agglutination is partially obscured by plasmolysis, and at  $pH$  5.2 the bacterial contents are reduced to granules. Similarly agglutination increases with increasing alkalinity until plasmolysis sets in.

If the reactions described above are not due to unnatural laboratory conditions, or to the methods by which the precipitate is obtained, but do actually occur in nature, some light seems to be thrown on the susceptibility of the potato to certain bacterial species whose "optimum"  $pH$  has been shown to be that of the potato sap, and on its resistance to attack by forms whose presence in cuts and wounds must be of common occurrence.

Laurent<sup>(9)</sup>, as long ago as 1899, found that the susceptibility of carrots and potatoes to disease could be varied by the use of lime or phosphatic manures, and confirmed his observations on the effect of lime in the field by showing that slices of potato and carrot soaked in weak alkali were attacked by *B. coli* and other saprophytic bacteria. Erwin Smith throws doubt upon his methods, but it seems quite possible that the effect of the alkali is to reduce the  $H$ -ion concentration of the sap to some value nearer the degree of acidity favourable to these organisms.

#### CULTURAL EXPERIMENTS.

In order to test whether the growth of various species in the presence of certain known constituents of the sap, *e.g.* phosphates and metallic salts, could be controlled in the same manner by varying the  $H$ -ion concentration, the growth of the fourteen types of bacteria was tested in Uschinsky's synthetic medium brought to the  $pH$  values 4.3, 4.6, 5.2, 5.5, 5.8, 6.4, 6.9, 7.4, since this medium contains besides asparagin, phosphates and small quantities of salts of di- and tri-valent metals.

Growth of the bacteria was slow, especially in the case of *B. delphinii* and *B. phytophthorus*, since the medium is poor in nitrogenous material. Turbidity usually only appeared on the second day, but by the fourth day the growth, if any, was well established; the tubes were kept for ten days at 20° C.

The results are summarised in columns 3 and 4 of Table I; column 3 gives the highest  $pH$  at which no growth occurred, and column 4 that at which growth was observed by the fourth day: the point at which inhibition of growth begins for each species lies between these two values. Column 2 is added for comparison; it gives the highest  $pH$  at which signs of plasmolysis were observed in the films made from the acidified potato precipitate solution. It will be seen that the maximum degree of acidity tolerated by each species in Uschinsky's solution lies near the  $pH$  at which acid plasmolysis of the organism was observed in the presence of the potato precipitate. The inhibition of growth in the Uschinsky cultures must be directly or indirectly due to the phosphates present, for omission of the metallic salts made no difference to the results. Plasmolytic effect on species with high "optimum" values, like *B. coli*, could moreover be observed in films made from agglutination tests with mixtures of K and Na acid phosphates in 0.1 per cent. solution. It seems probable therefore that the phosphates precipitable from the potato juice by the 95 per cent. alcohol, or rather the H-ion associated with them, is responsible for the plasmolysing and inhibiting action of the juice on those species whose optimum  $pH$  is higher than the  $pH$  of the sap itself, such as *B. coli*, *B. vulgare*, *B. mycoides* and *B. dendroides*. The immunity of the potato from attack by such organisms seems therefore to be dependent on the presence of phosphates in the sap; their action, however, may be reinforced by the plasmolytic constituent which is not precipitated by alcohol, but which is very active in the fresh juice.

The immunity of the potato from attack by species of bacteria with lower "optimum"  $pH$  values than 6.0 could not be attributed to the presence of phosphates in the sap. There was no inhibition of growth of these species in Uschinsky's solution at  $pH$  6.2, 6.9, 7.4, which would correspond with the agglutinating effect of the potato precipitate on them at these values. Potassium and sodium phosphates, like other salts of these elements, have little agglutinating power on bacteria. If, however, traces of metallic salts such as manganese acetate or calcium chloride in a dilution of about 0.001 were added to the phosphate solutions, the films made after 4 hours' agglutination tests showed marked flocculation, especially with fluorescent types when the reaction of the solution was adjusted to  $pH$  6.0-6.4 with NaOH; large patches of clustered bacteria were formed, especially in the manganese solutions, which were quite similar to those seen in films made from potato precipitate solutions (Fig. 2); *B. solanisaprus* and *B. carotovorus* on the other hand were hardly affected at all.

The agglutination due to the potato precipitate, whether it be due to traces of metallic salts or to other constituents, may not itself cause appreciable inhibition of growth, as agglutination is known to be a transient effect, but it may be a necessary preliminary to the action of some agent in the juice which is not precipitated by alcohol, for Mines (12) has shown that "neutral solutions of simple tri-valent ions quickly cause a marked alteration in the ionic permeability of a membrane." The more agglutinable forms like *B. fluorescens* may therefore be especially sensitive to bactericidal constituents of the potato sap not present in the precipitate.

On the other hand, possibly the mere fact that the mobility of the fluorescent forms is checked by agglutination during the period while incipient healing processes are taking place in the wound, may be sufficient to turn the scale in favour of the plant when invaded by this type of bacterium.

#### SUMMARY.

1. The fresh juice of potato is shown to possess agglutinating action on certain bacteria, and strong plasmolytic power which is gradually lost on exposure to the air.

2. The agglutinating power is found to be due to substances which are precipitated from the potato juice by 95 per cent. alcohol, and is independent of any previous inoculation of the potato with bacteria.

3. At the natural H-ion concentration of the potato sap the precipitate solution agglutinates and plasmolyses non-pathogenic species to potato in varying degrees, but the pathogenic species tested were unaffected by it.

4. The agglutinating power of the potato precipitate for any one species varies with the H-ion concentration of the solution; each species remains unagglutinated and unplasmolysed at one definite H-ion concentration.

5. This point of non-agglutination at which the species appears to be least affected by the potato precipitate, was found to be approximately the H-ion concentration of the sap of the potato itself, i.e. about pH 6.2, in the case of the two species causing soft rot in the potato. The twelve species non-pathogenic to potato which were tested have non-agglutination points above or below this.

6. The precipitate has a plasmolytic as well as an agglutinating action on any species at pH values more acid than its point of non-agglutination; this has been shown by cultural experiments to be probably dependent on the presence of phosphates in the precipitate.

7. The agglutinating power may possibly be due to traces of salts of di- and tri-valent metals in the precipitate.

The writer wishes to record her indebtedness to Prof. V. H. Blackman and to Dr S. G. Paine for the laboratory facilities accorded her, and for helpful interest and criticism in the preparation of this paper. Especially her thanks are due to Dr Paine for the two photographs illustrating agglutination.

#### REFERENCES.

- (1) BECHHOLD, H. (1904). Die Ausflockung von Suspensionen, bzw. Kolloiden und die Bakterienagglutination. *Zeitsch. f. physik. Chemie*, XLVIII, 385.
- (2) BERRIDGE, E. M. (1924). The influence of H-ion concentration on the growth of certain bacterial plant parasites and saprophytes. *Ann. of App. Biol.* XI, 1.
- (3) CLARK, W. M. (1920). *The determination of H-ions*. Williams and Wilkins, Baltimore.
- (4) CLEVINGER, C. B. (1919). The H-ion concentration of plant juices. *Soil Science*, VIII, 217.
- (5) EISENBERG, P. (1919). Ueber säure Agglutination von Bakterien, und über chemische Agglutination im Allgemeinen. *Centralbl. f. Bakt.* I Abt. (Orig.) LXXXIII, 561.
- (6) KORINEK, J. (1924). *Au sujet des agglutinines spécifiques chez les Végétaux*. Publ. de la Fac. des Sci. de l'Univ. Charles, Prague.
- (7) KRITCHEWSKY (1922). Ueber bakterielle Agglutinine und Praecipitine vegetabilischer Herkunft, im Zusammenhange der Pflanzen Immunitäts-körper zu produzieren. *Res. Centralbl. für Bakt.* II Abt. LII.
- (8) LACEY, M. S. (1926). Studies in bacteriosis. XIII. A soft rot of potato tubers due to *B. carotovorus*, and a comparison of the cultural, pathological, and serological behaviour of various organisms causing soft rots. *Ann. App. Biol.* XIII, 1.
- (9) LAURENT, M. E. (1899). Recherches expérimentales sur les maladies des plantes. *Ann. de l'Inst. Pasteur*, I, XIII.
- (10) MARTIN, S. H. (1927). The hydrion concentration of plant tissue. III. The tissues of *Helianthus annuus*. *Protoplasma*, I, Heft 4.
- (11) MEHTA, M. and BERRIDGE, E. M. (1924). Studies in bacteriosis. XII. *B. pyocyaneus* as a cause of disease in lettuce, and the identity of *B. marginale* with this organism. *Ann. of App. Biol.* XI, Nos. 3 and 4.
- (12) MINES, G. R. (1911). The action of tri-valent ions on living cells and on colloidal systems. II. Simple and complex kations. *Journ. Physiol. Chem.* XLII, 309-31.
- (13) REA, M. W. and SMALL, J. (1927). The hydrion concentration of plant tissues. V. The tissues of *Vicia faba*. *Protoplasma*, II, Heft 1.
- (14) SCHIFF-GIORGINI, R. (1906). Untersuchung über die Tuberkul-Krankheit des Oelbaumes. *Centralbl. f. Bakt.* II Abt. xv.
- (15) WAGNER, R. J. (1915). Ueber bakterizide Stoffe in gesunden und kranken Pflanzen. *Centralbl. f. Bakt.* II Abt. XLII.
- (16) YOUNDEN, W. J. and DENNY, F. E. (1926). *Factors influencing the pH equilibrium known as the iso-electric point of plant tissue*. Contrib. from the Boyce Thompson Inst. for Plant Research, I, No. 4.

(Received February 1st, 1929.)

# THE MORPHOLOGY AND PHYSIOLOGY OF TWO LACTOSE-FERMENTING YEASTS AND CHEMICAL CHANGES DURING THE RIPENING OF CHEESE FROM MILK CONTAINING THEM

By L. A. ALLEN, B.Sc. AND B. D. THORNLEY, B.Sc.

(From the Bacteriological and the Biochemical Laboratories of the Imperial College of Science and Technology, London.)

(With 4 Text-figures.)

## CONTENTS.

	PAGE
I. INTRODUCTION . . . . .	578
II. DESCRIPTION OF THE YEASTS UNDER INVESTIGATION . . . . .	579
Morphological characters . . . . .	579
Cultural characters in various media . . . . .	580
III. COMPARISON OF THE COLONIES AND RATE OF GROWTH OF <i>B</i> AND <i>C</i> AND THEIR TEMPERATURE RELATIONS . . . . .	582
Thermal death point . . . . .	583
Composition of gas evolved during fermentation . . . . .	583
Giant colonies . . . . .	584
IV. COMPARISON OF YEASTS <i>B</i> AND <i>C</i> WITH OTHER LACTOSE-FERMENTING YEASTS . . . . .	585
V. ACTION OF THE TWO LACTOSE-FERMENTING YEASTS ON THE PROTEIN AND CARBOHYDRATE OF MILK . . . . .	585
VI. EFFECT ON THE LACTOSE . . . . .	588
VII. EFFECT ON CHEESE MADE FROM MILK INOCULATED WITH YEASTS <i>B</i> AND <i>C</i> . . . . .	590
VIII. CHEMICAL COMPARISON OF TWO RIPE CHEESES . . . . .	591
IX. DISCUSSION OF RESULTS . . . . .	593
X. SUMMARY . . . . .	594
REFERENCES . . . . .	594

## I. INTRODUCTION.

Two yeasts isolated from gassy cheese at the National Institute for Research in Dairying, Reading, were found to possess the power of fermenting lactose, and, since little is known of the chemical behaviour of lactose-fermenting yeasts or of the part they play in the ripening of cheese, this investigation was undertaken.

Search of the literature shows that several lactose-fermenting yeasts and torulae have, from time to time, been discovered in milk and milk products, probably the first being that of Grotenfelt (1889) described under the name of *Saccharomyces Lactis Acidi*, while others were added by Adametz (1889), Beijerinck (1889), Kayser (1891), Freudenreich and Jensen (1897), Jørgensen (1898), Harrison (1902), Jensen (1902), Mazé (1903), Dombrowski (1910), Hunter (1918) and Hammer and Cordes (1920). Dombrowski studied a variety of yeasts isolated from milk and other products, including Mazun, Yoghourt, Kefir, cream and a milk starter. Of 26 cultures so obtained 10 fermented lactose and none fermented maltose. Of the lactose-fermenters four were *Saccharomyces* species, as evidenced by spore formation, and the remainder were *Torulæ*. Hunter described a lactose-fermenting yeast responsible for foamy cream. The organism was oval in shape, averaging  $5\mu$  by  $2\mu$  in size, did not form spores and had an optimum temperature for growth at  $37^{\circ}\text{C}$ . or higher.

Dombrowski and Hunter review the literature cited above. There therefore seems no useful purpose to be served by extending a discussion of it here.

## II. DESCRIPTION OF THE YEASTS UNDER INVESTIGATION.

For the purposes of this description the yeasts will be designated as *B* and *C*.

*Morphological characters.* Preparations made from cultures some months old and stained with cold carbol fuchsin showed the cells to be mainly oval, with occasionally elongated and sausage-shaped forms. Subcultures from beerwort agar (48 hours) showed much less tendency to elongation. Budding was evident in both *B* and *C*. Measurement of the unstained cells from a 48-hour culture on beerwort agar gave the following dimensions (an average of 100 measurements in each case):

<i>B</i>	Average ...	...	$4.5\mu$ by $3.6\mu$
	Maximum	...	$6.7\mu$ by $5.1\mu$
	Minimum	...	$2.9\mu$ by $2.4\mu$
<i>C</i>	Average ...	...	$5.0\mu$ by $3.8\mu$
	Maximum	...	$8.6\mu$ by $5.2\mu$
	Minimum	...	$2.9\mu$ by $2.5\mu$

Ascospores were formed in *B* as the result of an isogamic fusion, usually resulting in the formation of four spores which were variously disposed in the two gametes. The shape of the ascus varied considerably

but was usually of a dumb-bell appearance. One or two cases were noted in which only two spores appeared. No spores were formed in *C*. It must therefore be recognised as distinct from *B* although in many respects it behaved similarly in culture (Fig. 1).

*Cultural characters in various media.* A pure culture of each yeast was first obtained by the dilution method (Paine, 1927). For a study of the cultural characters sugar broths and solid media were used. Incubation was at 30° C. unless otherwise stated. Sugar broths consisted of a



Fig. 1. Appearance of ascospores in *B*.

solution of peptone 1 per cent., common salt 0.25 per cent. and sugar 1 per cent., adjusted to a *pH* of 7.3 before sterilisation.

The schedule following describes briefly the appearance on the various media and the biochemical characters exhibited by the two yeasts. Since on many media the yeasts *B* and *C* gave identical growth the description has been arranged thus:

*Group 1.* Growth characteristics on media showing no distinction between the two yeasts.

*Group 2.* Growth characteristics on media showing noticeable differences between the two yeasts.

### *Group 1.*

#### *Solid media:*

*Gelatin stab.* Growth slow, following the line of inoculation. Consisted of small, isolated, spherical colonies presenting the appearance of minute bubbles. Small, round, moist surface colonies. No liquefaction. Incubation at 20° C.

*Gelatin plate.* Small, round, white, wet-shining colonies. No liquefaction. Incubation at 20° C.

*Gelatin streak.* Very thin, dry, white, translucent growth. Had the appearance of a dry film on the gelatin. Incubation at 20° C.

*Agar plate.* White, wet-shining, raised colonies in 48 hours. Later colonies opaque with translucent edges. *C* was of noticeably slower growth than *B*. Slight fruity odour.

*Agar streak.* Thin, rather dry, yellowish-white growth in 24 hours. Growth moist but sparse after 3 days. Margin well defined though little growth in its vicinity.

*Beerwort agar streak.* Copious, greyish-white growth in 24 hours. Thick and continuous after three days, with a raised margin. After several days the margin had spread outwards in a smooth, slanting growth. In the case of *C* the margin was more serrated than in *B*. There was a strong fruity odour in both cases.



*Separated milk agar plate.* } The growth characteristics here were the same as  
*Separated milk agar streak.* } for the corresponding plates on beerwort agar.

*Sugars:*

*Glucose.* Acid with white sediment but no gas after 24 hours. After 48 hours small bubbles of gas formed. After three days gas increased considerably. After four days gas decreased to small bubble. After six weeks still slightly acid but gas had disappeared; yellowish-white sediment.

*Lactose.* Acid and gas formed after 48 hours, in larger quantity than in the case of glucose. Gas disappeared after six weeks and liquid was neutral in reaction.

*Sucrose.* Acid after 24 hours. Acid with gas formation after three days. Gas disappeared and liquid was neutral in reaction after six weeks.

*Galactose.* Acid after 24 hours. Formation of gas after three days; white sediment. After six weeks gas disappeared, medium was slightly alkaline and rather turbid; yellowish-white sediment.

*Levulose.* Acid with white sediment after 24 hours. Formation of gas after 48 hours. After four weeks still acid with yellowish-white sediment. After six weeks gas had disappeared while the medium was slightly alkaline and rather turbid.

*Maltose.* Very slightly acid after 48 hours. No gas. After several days distinctly alkaline and rather turbid. After seven weeks condition unchanged.

*a Methyl glucoside.* No change after 24 hours. Slightly acid with slight sediment after 48 hours. No gas. After several days medium became alkaline. After six weeks still alkaline and rather turbid.

*Raffinose.* No change for some days. Medium then gradually turned alkaline with formation of slight white sediment. After six weeks still alkaline and rather turbid.

*Various broths:*

*Mannite.* Slightly acid after 24 hours. After four days still slightly acid; no gas. After six weeks still acid; medium turbid with yellowish-white sediment. After five weeks neutral. After seven weeks alkaline.

*Dextrin.* Slightly acid after 24 hours. After several days medium quite alkaline. After six weeks strongly alkaline. Yellowish-white sediment.

*Peptone water.* Slight white sediment after 24 hours. After six weeks fairly thick white sediment. Liquid clear; no pellicle; no ring.

*Nitrate.* Slightly alkaline after several weeks. Otherwise no change.

*Uchinsky solution.* Very slightly alkaline after several weeks. Otherwise unchanged.

*Litmus milk.* Slightly acid after 24 hours. After 48 hours evolved considerable quantities of gas on shaking. Noticeable odour of alcohol. No further change after several weeks. Milk not curdled.

*Group 2.*

*Beerwort agar plate:*

*B.* Silky white, round, wet-shining colonies in 48 hours. Moist-shining flattened colonies with dense centre after three days. After five days the centre was yellowish-brown with a white margin. After two weeks the colonies were quite large and the centre was more deeply tinted than the margin. Later the centre turned a chocolate colour and consisted of several concentric rings. A very pronounced pungent fruity odour was noticeable which gradually disappeared with age.

*C.* Minute, white, round, wet-shining colonies after 48 hours. After several days they became more raised than *B*, of a creamy colour, and marked in sectors. The same creamy colour was preserved for some weeks. The same pronounced fruity odour as in *B* was observed. The chocolate colour associated with *B* was a somewhat variable characteristic. Sometimes it was of quite a deep brown colour and at other times only a light brown on the same medium. The concentric rings in the colonies of *B* and the sectors in the case of *C* grew more pronounced with age and were most noticeable in the case of giant colonies incubated at a low temperature and kept for three or four months.

*Beerwort:*

*B.* Very slight white ring formed at the surface after 24 hours. After four days the ring was fairly thick and there was a white sediment and a fruity odour. Considerable gas was evolved on shaking. After two weeks the ring was quite thick with a slimy appearance.

*C.* No apparent change in 24 hours. After several days there was a thick white sediment and a fruity odour. Gas evolved on shaking. After two weeks there was usually no further change, but occasionally a thin white ring was observed.

In the case of *B* the ring was always observed, while in the case of *C* there was usually no ring at all.

### III. COMPARISON OF THE COLONIES AND RATE OF GROWTH OF *B* AND *C* AND THEIR TEMPERATURE RELATIONS.

A suspension of each yeast was made in sterile water and single point inoculations of both made on the surface of beerwort agar in the same Petri dish. Four dishes were prepared in this way and were incubated at 20, 25, 30 and 37° C. respectively. The colonies were examined and the relative rate of growth examined by measurement of their diameters. At 20° C. and 25° C. very little difference in the appearance of the colonies could be detected. Both *B* and *C* produced round, white, flat colonies with a dull, moist centre and a shiny translucent margin. At 30° C. differences were noticeable and have been recorded below. At 37° C. *B* grew very slowly and after several days produced a small round colony with a light yellow centre and a grey margin. *C* would not grow at 37° C. Incubation at this temperature therefore provided a means of distinction between the two yeasts.

The results of measurements at various temperatures are shown below:

		20° C.	25° C.	30° C.	37° C.
24 hours	<i>B</i>	No growth	1.5 mm.	3.0 mm.	No growth
	<i>C</i>	"	1.5	1.0	"
3 days	<i>B</i>	3.0 mm.	6.0	9.5	1.5 mm.
	<i>C</i>	2.5	6.0	6.5	No growth
7 days	<i>B</i>	5.0	8.0	13.0	2.5 mm.
	<i>C</i>	4.5	8.0	8.5	No growth

It will be seen that for the first 24 hours the optimum temperatures are 30° C. for *B* and 25° C. for *C*. After that the optimum temperature for both is 30° C. *C* was always of slower growth than *B* on all the media tested. Moreover *B* grew much more quickly at 30° C. than at 25° C. while *C* grew equally well at both temperatures.

*Thermal death point.* 48-hour cultures in beerwort were used and a period of 10 minutes taken as the standard of time required to cause

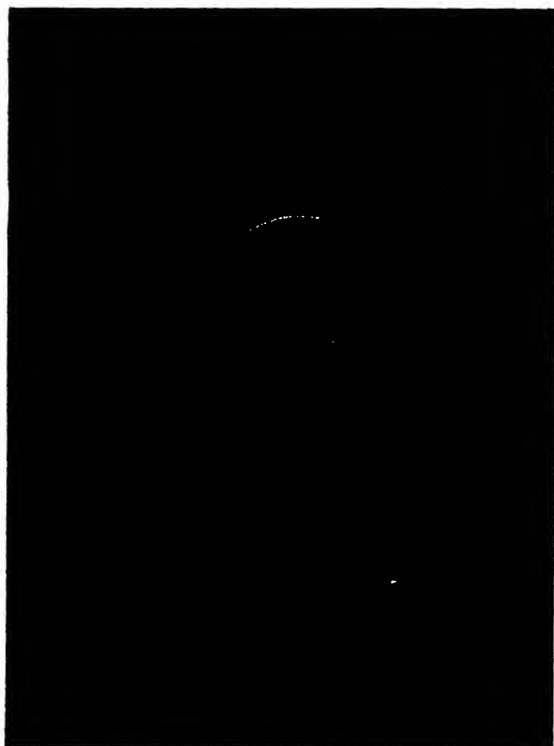


Fig. 2. Giant colony, *B*.

death. The thermal death point of *B* was found to be between 52° C. and 54° C. and of *C* between 50° C. and 52° C.

*Composition of gas evolved during fermentation.* Inoculations were made into Einhorn Saccharimeter tubes filled in one case with beerwort and in the other with 1 per cent. lactose broth. In each case the gas evolved by *B* and *C* was entirely absorbed by strong caustic soda. Carbon dioxide was therefore the only gas produced during fermentation.

*Giant colonies.* Single point inoculations of *B* and *C* were made on beerwort agar and incubated at 10–12° C. for 16 weeks. Growth was very slow and very little difference could be detected in the characters of the colonies for three or four weeks, but after two months the differences were well defined. Since these giant colonies provided a means of distinction between the two yeasts a detailed description of their appearance after four months is of interest:

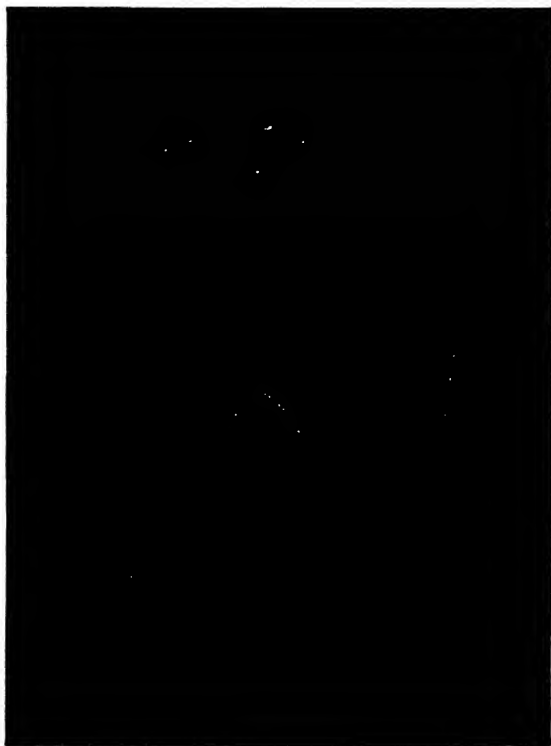


Fig. 3. Giant colony, *C*.

*B.* Light brown colour except for  $\frac{1}{4}$  in. at the edge which was of a creamy colour, and the extreme margin, which was grey and translucent. The centre was raised and of a dull "frosted" appearance to a diameter of about 1 in. Outside that the colony was smooth to the edge and consisted of four thin, concentric bands, surrounded by a system of radial lines, some broken and some entire, giving a crinkled appearance (Fig. 2).

*C.* Rather larger than *B* and comparatively smooth in appearance,

the centre not being raised. The central portion was of a slight yellowish-brown tint. The colony was divided into two sectors, one sector being well defined and of a somewhat different appearance from the remainder. It was smooth and consisted of thin, concentric bands from the centre to the edge. The remainder was of a shiny "frosted" appearance to within  $\frac{1}{2}$  in. of the edge, where it was quite smooth with thin, concentric, wavy bands, ending in a grey, translucent, wavy margin. There were a few radial lines not reaching to the centre (Fig. 3).

#### IV. COMPARISON OF YEASTS *B* AND *C* WITH OTHER LACTOSE-FERMENTING YEASTS.

Two strains of lactose-fermenting yeasts were available for comparison and were obtained from the Collection of Type Cultures at the Lister Institute, London. One of these was *Saccharomyces fragilis* Jørgensen and the other a yeast which had been isolated and presented by Thaysen. Both proved to have many characteristics in common with *B* and *C* but were distinguished from *B* by the absence of sporulation, and from *C*, *S. fragilis* differed markedly in shape, the cells being more elongated and the prevailing form sausage-shaped. Thaysen's organism was considerably larger, averaging  $6.5\mu$  by  $4.8\mu$  and was characterised by an optimum temperature for growth in the neighbourhood of  $45^{\circ}\text{C}$ . The appearance of its giant colony was also markedly different.

Reference to the description given by Dombrowski (1910) of his new species *Zygosaccharomyces lactis* shows that yeast *B* agrees in many respects with this species, for instance in its copulation and spore formation, size of cells, sugar fermentations and absence of milk coagulation, so, although Dombrowski's description is a little brief, there seems every probability that yeast *B* belongs properly to this species. Yeast *C* is properly termed a *Torula* and its description has been rather fully given in this paper so that others who follow may know what class of organism the authors had before them. It in all probability is to be identified with one of the earlier discovered lactose-fermenting torulae; the descriptions of these, however, are too meagre to enable one to decide between them.

#### V. ACTION OF THE TWO LACTOSE-FERMENTING YEASTS ON THE PROTEIN AND CARBOHYDRATE OF MILK.

The object of the first series of experiments was to discover whether the yeasts *B* and *C*, though they could not be classed as liquefiers of gelatin, had the power after a lengthy period of incubation to cause

a breakdown of the casein in milk. For this purpose fresh separated milk was used. Flasks containing 90 c.c. and tubes containing 10 c.c. were plugged and sterilised by intermittent steaming. The quantities of 10 c.c. were now inoculated, some with *B* and some with *C*, by transfer from a 48-hour culture on beerwort agar. These were incubated at 30° C. for four days to ensure a vigorous growth and were then poured under aseptic conditions into the flasks, the total volume in the latter being 100 c.c. In this way, by commencing with a sufficiently large and vigorous inoculum, good growth of the yeast in the comparatively large volume of milk (100 c.c.) was ensured. Cultures so prepared were incubated at 20° C. At intervals for a period of 16 weeks the extent of protein degradation in these cultures was measured by Sørensen's method of formol titration. The contents of a flask were well whisked to break up any deposited sediment and transferred quantitatively to a 250 c.c. graduated flask, the liquid being made up to the mark with distilled water. The contents were well shaken to ensure uniform distribution and an aliquot portion abstracted with a pipette. This was boiled for three minutes to expel CO<sub>2</sub> and titrated with decinormal sodium hydroxide, using phenol-phthalein as an indicator. Neutralised formalin was now added and the mixture again titrated to neutrality. The first reading then gave a measure of the acidity, and the difference between the first and second readings gave a measure of the peptide scission. Results were compared with those obtained for similar flasks of uninoculated milk kept under the same conditions.

The following tables show the effects observed. Table I represents the increase in acidity in terms of decinormal alkali, and Table II the increase in formol titration.

Table I.

*Increase in acidity per  
100 c.c. milk.*

Time	c.c. of N/10 NaOH	
	<i>B</i>	<i>C</i>
1 week	3.36	3.46
2 weeks	18.55	13.93
4 "	17.14	17.44
9 "	17.85	18.05
13 "	17.32	—
15 "	15.19	14.97

Table II.

*Increase in formol titration  
per 100 c.c. milk.*

Time	c.c. of N/10 NaOH	
	<i>B</i>	<i>C</i>
1 week	0.37	1.06
2 weeks	0.19	1.55
4 "	0.57	2.22
9 "	5.23	7.95
13 "	9.84	—
15 "	12.64	17.13

It will be seen that, broadly speaking, the acidity increased until the end of the second week, or shortly after, and then remained constant for some weeks. After a rather lengthy period the acidity appeared to decrease. Since, as will be shown later, all the lactose was used up after about 14 to 17 days it is fairly safe to infer that the acid was a decomposition product of the lactose. Tests for lactic acid with Uffelman's solution failed to give positive results. For the first two weeks there

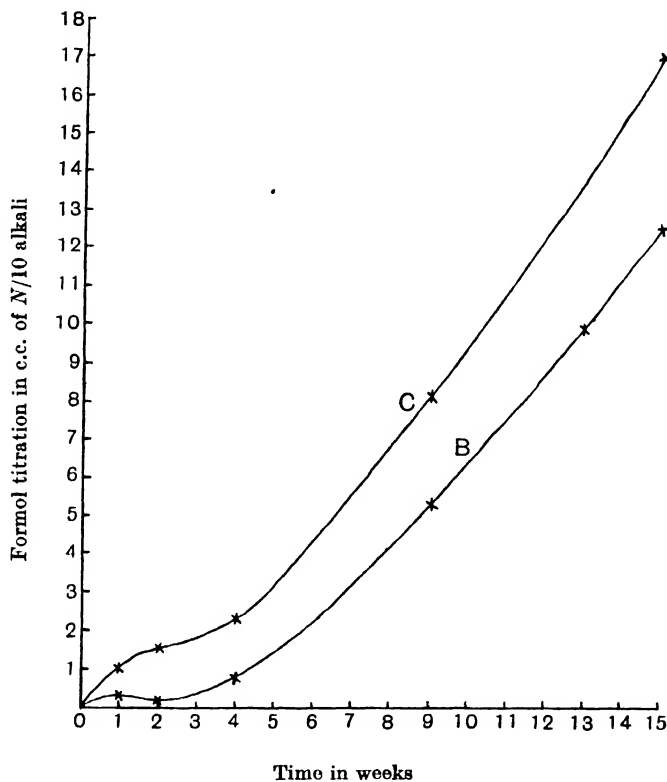


Fig. 4. Curves showing rate of protein degradation in milk by the yeasts *B* and *C*.

was certainly no appreciable increase in the formol titration and after four weeks the amount was very small, so that protein decomposition was not simultaneous with that of the lactose. After 9 weeks there was an appreciable peptide scission, and after 15 weeks a fairly considerable amount. From beginning to end *C* appeared to have a greater effect on the proteins than *B*, and at the end of the fifteenth week the difference was quite marked.

Similar experiments were made with milk inoculated with *Streptococcus lacticus* and with milk inoculated with a mixture of *Streptococcus lacticus* and the yeast *B*. In the former case inoculation was made direct into quantities of 90 c.c. of milk and the flasks were incubated at 37° C. for 18 hours before incubating for the period of the experiment at 20° C. In the case of the mixed culture the yeast was first inoculated into 10 c.c. of sterile milk and incubated for three days at 30° C. The *Streptococcus lacticus* was meanwhile inoculated into the 90 c.c. of milk in the flasks, and after 18 hours' incubation at 37° C. the culture of *B* was poured in under aseptic conditions and the flasks incubated at 20° C. In this way some attempt at comparable conditions was made, for a slow-growing yeast could not well compete with a fast-growing *Streptococcus* unless first made into a vigorous inoculum.

The results of these experiments showed that at the end of 10 weeks no protein degradation had occurred in either series. Moreover at the end of that period there was still a considerable quantity of lactose remaining in both sets of cultures.

## VI. EFFECT ON THE LACTOSE.

A series of experiments was next made to discover the relative rate of decomposition of the lactose in milk of (a) the yeast *B* alone, (b) the *Streptococcus* alone, and (c) a mixture of the yeast and *Streptococcus lacticus*. For this purpose cultures in flasks were prepared as in former experiments, one set being inoculated with a 48-hour culture of the yeast, a second set inoculated with *Streptococcus lacticus* and incubated at 37° C. for 18 hours before incubating at 20° C., and a third set treated in exactly the same way as the second set and then inoculated with 10 c.c. of a 48-hour culture of *B* before incubating at 20° C. Lactose determinations were made on the three sets of cultures at frequent intervals for 16 days and then at less frequent intervals. The contents of a flask were well whisked as before and made up to a volume of 250 c.c. It was found that a satisfactory homogeneous emulsion was obtained by vigorous shaking and an aliquot portion easily abstracted for analysis. The protein and fat were precipitated with acid mercuric nitrate, filtered and thoroughly washed with warm water, and the lactose was determined in the filtrate by Bertrand's method.

The results of the experiments are shown in Tables III and IV where the first column in each represents the time after inoculation, the second column gives the percentage of lactose present in the milk



at that time, and the third column shows the percentage of the original lactose which had been decomposed at the corresponding time.

Table III.

*Yeast B.*

Time	% lactose present	% decomp.
0	4.73	0.00
30 hours	4.46	5.71
78 "	4.12	12.90
128 "	3.35	28.33
168 "	2.99	36.79
11 days	0.99	79.16
14 "	0.69	85.41
17 "	Trace	100.00
—	—	—

Table IV.

*Yeast + Strep. lact.*

Time	% lactose present	% decomp.
0	4.17	0.00
—	—	—
78 hours	3.87	7.19
128 "	3.76	12.23
168 "	3.51	15.83
10 days	3.33	20.14
14 "	3.28	21.34
8 weeks	1.47	62.02
10 "	0.28	93.28

In the experiments on the cultures of *Streptococcus lacticus* alone it was found that in the early stages of growth the lactose content varied so much from flask to flask that comparable results were impossible. In relation to the effects of the yeast and of the yeast plus *Streptococcus lacticus*, however, the rate of consumption of lactose in the cultures of the *Streptococcus* alone was, after an initial rapid growth, very slow. Thus after eight weeks only 14.01 per cent. of the lactose had been used up and the amount was increasing very slowly indeed.

It will be seen from these results that the yeast alone uses up the lactose far more quickly than the *Streptococcus* or a mixture of the yeast and the *Streptococcus*. The rate of consumption of the lactose for a mixture of the two may be said to be a mean of the rates for either of the two singly. This may indicate either that the *Streptococcus* considerably retards the growth of the yeast or that the yeast feeds on the decomposition products of the lactose which are provided by the *Streptococcus*. The latter idea pointed the way to an experiment to determine whether either of the yeasts could utilise lactic acid as a food. For this purpose a medium containing 0.3 per cent. meat extract, 0.25 per cent. NaCl, 1.0 per cent. peptone and 1 per cent. lactic acid was prepared, transferred in quantities of 10 c.c. to tubes and sterilised in the autoclave at 115° C. for 20 minutes. Some tubes were then inoculated with yeast *B*, some with yeast *C* and some left as control, the whole being incubated at 30° C. After several days the cultures of *B* and *C* showed turbidity and a slight white sediment indicating growth. The lactic acid present was determined by boiling the liquid for three minutes to expel CO<sub>2</sub> and

titrating to phenol-phthalein with decinormal sodium hydroxide. Results are shown in the following table:

Time	c.c. of <i>N</i> /10 NaOH	
	<i>B</i>	<i>C</i>
0	10.11	10.11
7 days	9.54	9.65
17 „	8.91	8.90

These indicate a small but definite decrease in titratable acidity which may reasonably be assumed to show a decrease in the lactic acid content. *B* and *C* would appear then to be able to utilise lactic acid as a food.

#### VII. EFFECT ON CHEESE MADE FROM MILK INOCULATED WITH YEASTS *B* AND *C*.

Two sets of four 10 lb. cheeses (English Cheddar) were made, the cheeses in each set being from the same milk. In one set two cheeses were inoculated with yeast *B* and two left as control. In the other set two were inoculated with yeast *C* and two left as control. Inoculation was made by adding to the vat two test-tubes full of a three-day culture of the yeast incubated at 30° C., and leaving overnight until the morning's milk was added. The cheeses were examined from time to time throughout the course of ripening and the inoculated ones compared with the control. After the first week the inoculated cheeses exhibited a "blown" appearance due to the evolution of CO<sub>2</sub>. Samples were taken by boring from the outside to the centre with a circular borer. Those from the inoculated cheeses were of a noticeably darker appearance, with a fruity odour which became more pronounced on mashing with warm water. The flavour was bitter and very yeast-like and unpleasant, while the texture was more open than the control cheeses and in consequence a portion was found to be more friable on grinding with sand in a mortar. The fruity odour disappeared after about three weeks, and the "blown" appearance of the cheeses was less and less noticeable until, at the end of four months, they had a normal appearance.

Attempts were made throughout the course of ripening to determine whether there was any distinct difference in the extent of protein degradation. For this purpose about two grams of cheese, taken from a sample boring, were well ground with fine sand, made into a suspension with water and kept at a temperature of 55° C. for half an hour. Formol titrations were then made in the same way as described for the milk. It was found, however, that results varied so much in the same cheese,

according as the samples were taken from the inner or outer portions, that comparable results between two cheeses were impossible. After five months the cheeses were cut and examined. The colour of the inoculated and control cheeses was practically the same, but the texture of the former was more open and the flavour was distinctly different. The unpleasantness of the inoculated ones had disappeared, but there was still a slight fruity flavour although the bitterness was absent.

#### VIII. CHEMICAL COMPARISON OF TWO RIPE CHEESES<sup>1</sup>.

A chemical comparison of two cheeses, one inoculated and one control, was made after eight months' ripening. For this purpose two whole cheeses, one control and one inoculated with yeast *C*, were sampled by grinding about a kilogram of each on a cheese grater and thoroughly mixing to ensure representative sampling. The moisture, fat and nitrogen contents were then determined in duplicate in the case of each cheese. For the moisture determination about five grams of the ground cheese were weighed and dried in a vacuum desiccator for four days. At the end of that time the remaining moisture was driven off in the steam oven, the process taking 24 hours. Fat was determined by extraction with ether by Soxhlet's method. Nitrogen was determined by Kjeldahl's method on about a gram of the ground cheese. For investigation of the decomposition products of the proteins the fat-extracted material was used. For this purpose about 50 grams of the ground cheese were fat-extracted for five hours and the ether and moisture remaining were driven off by placing in an air oven at 55° C. for 24 hours. At the end of this time the product was hard and perfectly friable. It was therefore ground as finely as possible by passing through a mill and then rubbed up in a mortar. A suspension of the powder so obtained was made by well mixing with sterile distilled water, transferring to a sterile 500 c.c. flask and making up to the mark with sterile water. Sterile water and flask were used in order to minimise any protein decomposition due to bacterial contamination. The suspension was well shaken to ensure uniformity and two portions of 25 c.c. quickly pipetted off for nitrogen determination. The remainder of the suspension was left to stand for 16 hours in order to allow all soluble substances to pass into solution.

At the end of this time the suspension was again well shaken and two further portions abstracted for formol titration. The remainder of the suspension was filtered through coarse filter paper, the filtrate being of

<sup>1</sup> This work was done by one of us (L.A.A.) at the National Institute for Research in Dairying, Shinfield.

a clear colour. A nitrogen determination and formol titration were made on portions of 25 c.c. of the filtrate. 50 c.c. of the filtrate were run into 200 c.c. of neutralised absolute alcohol and the mixture made up to 250 c.c. by the addition of neutralised 80 per cent. alcohol. This was left for 24 hours and then filtered. A nitrogen-content determination and formol titration were made on aliquot portions of this filtrate.

In this way three sets of figures were obtained, viz. the nitrogen-content and formol titration for (1) the aqueous suspension, (2) the aqueous filtrate, (3) the alcoholic filtrate. In the case of the suspension all the decomposition products of the casein, from the protein itself to amino acids, would be present. In the aqueous filtrate casein and some of the higher decomposition products would be eliminated. The object of the 80 per cent. alcohol was to provide some means of partial separation of the amino acids and the lower polypeptides from the higher polypeptides and peptones. The alcoholic filtrate therefore contained the lowest decomposition products of the casein. For the formol titration on the suspension and aqueous filtrate Foreman's method as modified by Harris (1923) was used, phenol-phthalein and alcohol plus formalin being preferred to thymol-phthalein and alcohol alone, as giving a better end-point. This determination also gave a measure of the acidity of the liquid as well as of the extent of peptide scission. The nitrogen-content of the alcoholic filtrate was determined by means of a micro-Kjeldahl apparatus. 5 c.c. of the filtrate were evaporated (in hard glass incineration tubes on the water bath) with five or six drops of concentrated hydrochloric acid to a point just short of dryness, so as to eliminate all the alcohol, while at the same time leaving behind all the volatile bases. To the residue 1.5 c.c. of  $\text{H}_2\text{SO}_4$ , two drops of  $\text{CuSO}_4$  solution and a little  $\text{K}_2\text{SO}_4$  were added. This was incinerated and distilled from alkali into  $N/70$   $\text{H}_2\text{SO}_4$  in the usual way. An iodimetric titration was used to determine the quantity of  $N/70$  acid still remaining after distillation. For this purpose 5 c.c. of a 1 per cent. solution of  $\text{KI}$ , 1 c.c. of a 1 per cent. solution of  $\text{KIO}_3$ , and 2 c.c. of a 1 per cent. solution of soluble starch (saturated with  $\text{NaCl}$ ) were added to the distillate. After leaving for five minutes to allow complete separation of the iodine the mixture was titrated with  $N/70$   $\text{Na}_2\text{S}_2\text{O}_3$ .

### *Results of analysis.*

	Control cheese	Inoculated cheese
Moisture content ...	21.68 %	29.85 %
Nitrogen content ...	6.12	5.55
Fat content ...	39.44	33.44

*Protein degradation—Nitrogen distribution.*

	Control cheese	Inoculated cheese
Aqueous suspension ...	1.0000 gm. <sub>a</sub>	1.0000 gm.
Aqueous filtrate ...	0.3915	0.4537
Alcoholic filtrate ...	0.2187	0.2680

*Acidity and formol titrations (per gram of nitrogen present in each case).*

		Control cheese	Inoculated cheese
Aqueous suspension	Acidity	81.48 c.c.	72.27 c.c.
	Formol titration	75.31	138.54
Aqueous filtrate	Acidity	46.52	45.07
	Formol titration	70.64	109.90
Alcoholic filtrate	Acidity	134.6	138.5
	Formol titration	269.6	396.4

Results of acidity and formol titrations are expressed in each case as c.c. of *N*/10 sodium hydroxide required for neutralisation. The figures given are calculated per gram of nitrogen present in each case and therefore afford a basis for strict comparison.

## IX. DISCUSSION OF RESULTS.

These results show a definite difference between the two cheeses in the major analysis, in the acidity and in the protein decomposition products. The apparent differences in the fat and nitrogen-contents are explained of course by the difference in the moisture-content. This would seem to indicate a difference in the texture of the cheeses giving rise to a different degree of tenacity for the moisture originally present. The results of the acidity titrations on the aqueous suspensions show that the inoculated cheese is considerably less acid than the control cheese, a result which may be expected both on general grounds and from the experiments already conducted on milk, inoculated in one case with the yeast alone and in the other case with the yeast and *Streptococcus lacticus*. Thus since the yeast gives rise to an alcoholic fermentation with the production of very little acid it may be expected to inhibit to a corresponding extent the formation of lactic acid by the starter introduced into the cheese at the commencement. This is borne out by the experiments on milk where it was shown that the yeast uses up the lactose more quickly than does the *Streptococcus* alone. Moreover, there was some indication that the yeast is capable of utilising the lactic acid as a food and thereby decreasing the acidity.

The formol titrations are perhaps the most interesting results since they give an indication of the extent of protein degradation. Those for the aqueous suspension indicate a greater amount of general decomposition in the case of the inoculated cheese. Those for the aqueous filtrate and

the alcoholic filtrate, combined with the figures for the nitrogen distribution, show both that the lower degradation products are present in greater amount and that they are of a lower order of degradation in the case of the inoculated cheese. Thus both the nitrogen-content and the formol titration *per gram of nitrogen present* are considerably greater in each case for the inoculated cheese.

The general conclusion is that the presence of a lactose-fermenting yeast in a cheddar cheese produces a more open texture, decreases its acidity, produces esters which impart a distinctive flavour, and brings about a greater decomposition of the casein.

### X. SUMMARY.

Two lactose-fermenting yeasts isolated from cheese have been described and though markedly similar in many respects they have been referred to separate species, the one being a *Torula* whose close relationship could not be definitely established, and the other being a true yeast believed to be correctly identified with *Zygosaccharomyces lactis* (Dombrowski).

The two yeasts have been found to have an appreciable proteolytic effect on the casein of milk after several weeks. The effect of one of the yeasts alone and of the yeast plus *Streptococcus lacticus* on the lactose of milk has also been studied.

Cheddar cheese inoculated with the yeasts has been found to differ considerably in quality and texture from the control cheese. A comparative study of the extent of protein degradation in two ripe cheeses, one inoculated with the *Torula* and one control, has shown the proteolytic effect of the yeast in the course of ripening.

The authors wish to express their thanks to Dr S. G. Paine for his constant interest and help in this work, and to Mr A. T. R. Mattick for many valuable suggestions.

### REFERENCES.

- ADAMETZ, L. (1889). *Saccharomyces lactis*, eine neue Milchzucker-vergärende Hefeart. *Centralbl. f. Bakteriol.* v, 117.  
 BEIJERINCK, M. W. (1889). Die Lactase eine neue Enzym. *Ibid.* vi, 44.  
 BOCHICCHIO, NICOLA (1894). Ueber einen Milchzucker-Vergärenden und Käseblähungen hervorruhenden neuen Hefepilz. *Ibid.* Abt. 1, xv, 546.  
 DOMBROWSKI, W. (1910). Die Hefen in Milch und Milchprodukten. *Ibid.* Abt. 2, xxviii.  
 FREUDENREICH, ED. und JENSEN, ORLA (1897). Ueber den Einfluss des Naturlabes auf die Reifung des Emmenthalerkäses. *Ibid.* Abt. 2, iii, 545.

- GROTFENFELT, G. (1889). Studien ueber die Zersetzungen der Milch. *Centralbl. f. Bakteriologie*. Abt. 1, v, 607.
- HAMMER, B. W. and CORDES, W. A. (1920). A study of lactose-fermenting yeasts present in yeasty cream. *Iowa Sta. Research Bul.* 61.
- HARRIS, L. J. (1923). The titration of amino- and carboxyl-groups in amino acids, polypeptides, etc. *Proc. Roy. Soc. B*, XLV, 440-84 and 500-22.
- HARRISON, F. C. (1902). Bitter milk and cheese. *Centralbl. f. Bakteriologie*. Abt. 2, ix, 206-25.
- HUNTER, O. W. (1918). A yeast producing foamy cream. *J. Bact.* III, 293.
- JENSEN, ORLA (1902). Studien ueber das Ranzigwerden der Butter. *Centralbl. f. Bakteriologie*. Abt. 2, viii, 251.
- JØRGENSEN, A. (1911). *Microorganisms and fermentation*. Philadelphia, 371.
- KAYSER, E. (1891). Contribution à l'étude physiologique des levures alcooliques du lactose. *Ann. de l'Inst. Pasteur*, v, 395.
- MAZÉ, P. (1903). Quelques nouvelles races de levure de lactose. *Ibid.* xvii, 2.
- PAINE, S. G. (1927). A practical method of culture from a single bacterial cell. *Journ. Bact.* xiv, 6.
- RUSSELL, H. L. and HASTINGS, E. G. (1905). A Swiss cheese trouble caused by a gas-forming yeast. *Wis. Exp. Station Bul.* 128.

(Received March 8th, 1929.)

# INVESTIGATIONS ON *HETERODERA SCHACHTII*, SCHMIDT. IN LANCASHIRE AND CHESHIRE

## PART III. CERTAIN CORRELATIONS BETWEEN CROP YIELDS AND DEGREE OF INFESTATION

BY A. M. SMITH, B.Sc., PH.D., A.I.C.

(*Edinburgh and East of Scotland College of Agriculture*),

AND

HERBERT W. MILES, M.Sc., N.D.A.

(*Adviser in Agricultural Entomology, Manchester University.*)

(With 1 Text-figure.)

IN a previous publication<sup>(2)</sup> the technique of estimating the degree of infestation of *H. schachtii* in the field was examined and an attempt made to correlate significant differences with the intensity of "eelworm disease" as estimated by visual observation. Although the results indicated that in those areas where disease had been noted comparatively recently there was a positive association between cyst count and intensity of disease, it was felt that a more accurate computation of the latter, by measuring crop yield, was necessary before any definite conclusion might be drawn. Furthermore, the fact that, in areas where disease had been observed more than three or four years previously, the cyst counts did not bear a close relationship to the state of the crop, indicated that *H. schachtii* was at most only one factor in the causation of what is termed "eelworm disease" of potatoes. It was decided, therefore, to lay down a number of plots on affected ground, make observations throughout the growing season, weigh the produce and compare the yields with the original and final degree of infestation as measured by cyst counts.

### EXPERIMENTAL.

*Field.* Four series of plots, varying in size from 1/130 to 1/40 of an acre were laid down in different localities. The first three series were on peat and the fourth on peaty sand. The soil characteristics and farm practice, together with the notes on the first appearance of disease, have



already been fully described (2). The plots were sampled about the middle of January, 1928, and measurements made of cyst content and a number of chemical properties. About the middle of March, 16 of the 29 plots were treated with varying amounts of calcium carbonate to provide data for another object in view. All the plots received the same manurial dressing in May, prior to planting with potatoes. The variety employed was Great Scot and all the seed was taken from one consignment. Field notes were made throughout the growing season and the crops were harvested at the beginning of September, the weights being taken to the nearest pound. The plots were re-sampled in December and the same measurements made as on those samples of soil which were taken nearly twelve months previously.

*Laboratory.* The soil samples were allowed to reach an air-dry condition and then passed through a 2 mm. sieve. Cyst counts were made as described (*loc. cit.* p. 326) on the fine earth portion. For each sample determinations were made of (a) the pH electrometrically (3), (b) the "lime-requirement" by the Hutchinson and MacLennan method, and (c) the content of free carbonate by means of a Collin's calcimeter. The results have been collected in Table I.

## RESULTS.

*Field observations.* So far as could be gathered from observations made in the field, growth commenced quite normally, and little or no difference could be noted on the different plots. There was, however, a considerable number of misses associated directly with the fungus *Rhizoctonia solani* Kühn. By the middle of August it was quite obvious that all the crops were far below the average and that, in the series 1 and 3, they were, from the practical point of view, almost complete failures. In those cases the foliage had an unhealthy appearance and was so dwarfed that the drills were not completely covered. Series 2 was not quite so bad, but many poor patches were to be seen at irregular intervals. Series 4 was undoubtedly the best, but even there the crop was not up to standard.

*Laboratory measurements.* In Table I the limed and unlimed plots have been kept separate in order to facilitate comparison. Figures for the total yield of potatoes, including ware and chats, are given alongside the original, final and change in cyst count. The difference between the amount of calcium carbonate added and the original "lime-requirement" was closely associated with the final content of carbonate in the soil: since, however, the unlimed plots almost invariably showed an increase

Table I.  
Results for series of plots.

UNLIMED.						LIMED.					
No.	Cyst count per 10 c.c. soil			Yield cwt./A	% CaCO <sub>3</sub> Change	No.	Cyst count per 10 c.c. soil			Yield cwt./A	% CaCO <sub>3</sub> Change
	Before	After	Change				Before	After	Change		
1 AB	11.8	11.1	-0.7	36	Nil	1 A	12.5	11.7	-0.8	40	2.75
1 BC	14.1	12.7	-1.4	23	0.15	1 B	10.8	13.0	+2.2	30	1.36
1 CD	17.8	12.5	-5.3	26	0.18	1 C	18.8	9.8	-9.0	19	1.99
1 DE	11.5	14.9	+3.4	39	Nil	1 D	14.7	15.0	+0.3	30	1.22
2 AB	20.8	22.7	+1.9	76	0.10	2 A	21.7	26.8	+5.1	114	0.35
2 BC	17.8	27.4	+9.6	94	0.29	2 B	19.7	25.6	+5.9	60	2.48
2 CD	10.6	15.5	+4.9	57	0.05	2 C	16.0	20.3	+4.3	53	0.93
						2 D	6.6	12.1	+5.5	105	0.87
3 A 1	21.0	13.3	-7.7	26	0.76	3 A 2	21.0	11.5	-9.5	34	1.52
3 B 2	25.0	21.8	-3.2	14	0.04	3 B 1	25.0	19.9	-5.1	28	1.24
4 A 3	17.0	20.7	+3.7	134	0.04	4 A 1	17.0	16.4	-0.6	122	0.72
						4 B 1	14.8	19.5	+4.7	119	0.28
4 C 1	19.7	23.9	+4.2	117	0.01	4 C 2	19.7	18.4	-1.3	91	0.14
4 D 3	18.6	19.4	+0.8	86	0.11	4 D 2	18.6	17.7	-0.9	87	0.30
4 E 2	9.0	12.8	+3.8	81	0.07	4 E 3	9.0	11.9	+2.9	108	0.25
						4 B 2	14.8	18.2	+3.4	99	1.26

in content of carbonate, owing, presumably, to the accidental transfer of lime from the adjacent limed plots, the increase in percentage of calcium carbonate has been given throughout. In view of the heavy dressings of lime and the comparatively short space of time elapsing since their application, not much stress could be laid upon the final *pH* figures for the limed plots, whilst the *pH* of the unlimed plots did not alter materially. Consequently, the *pH* values have not been included.

The most striking feature of the results is the very low yields obtained from the diseased areas upon which the plots were laid. A normal yield fluctuates about 10 tons per acre, but on those plots the yields vary from less than 1 ton to about 6 or 7 tons per acre; the average yield is little more than 30 per cent. of the normal.

As a preliminary step towards the elucidation of any associations, scatter diagrams were prepared from the various pairs of values. It was evident that no close association existed between the yield and the amount of calcium carbonate present or between the yield and the original cyst concentration of the soil. There was an apparent negative association of values representing change in cyst count and original cyst count, and the same thing could be said to a less degree of the change in cyst count and the increase in calcium carbonate. The most obvious association, however, lay between yield of potatoes and change in cyst

count. An examination of the data shows that, with few exceptions, the cyst count has increased when the yield is more than 50 cwt. and decreased when the yield is less than 50 cwt. The figures for yield and change in cyst count are plotted in Fig. 1, and the general trend of the results is fairly obvious.

In order to examine the possible associations more closely, "total correlation" coefficients for six pairs of values have been calculated and are shown in Table II.

Table II.

*Coefficients of correlation between pairs of values.*

	<i>A</i>	<i>O</i>	<i>L</i>
<i>Y</i>	+0.592	-0.131	-0.315
<i>A</i>	—	-0.409	-0.232
<i>O</i>	—	—	+0.044

The letters *A*, *O*, *L*, *Y* designate, respectively, alteration in cyst count, original cyst count, increase in percentage of calcium carbonate and yield. The correlation between *A* and *Y* has been calculated from the formula (1),

$$r_{AY} = \frac{\Sigma ay}{n\sigma_a\sigma_y},$$

where  $\Sigma ay/n$  = the mean product of the deviations of *A* and *Y* from their means,

$\sigma_a$  = the standard deviation of *A*,

$\sigma_y$  = the standard deviation of *Y*.

For 29 sets of observations, the only correlation which is definitely significant is that between *A* and *Y*. The coefficient  $r_{AO} = -0.409$  has a probability between 0.05 and 0.02 and is, therefore, just significant.

The correlations between *A* and *Y* when *O* and *L* are eliminated are

$$r_{AY.O} = 0.596 \text{ and } r_{AY.L} = 0.562,$$

which show that the association between *A* and *Y* is independent of the original cyst concentration, and is reduced slightly by the elimination of *L*, the increase in calcium carbonate present. It is not possible to say whether *A* and *Y* vary independently or the extent to which they may be interdependent, but certain factors apparently affect *A* and *Y* similarly and to an important extent compared with other factors, so that, generally speaking, over the diseased areas investigated the cyst concentration increases in proportion to the yield when that is greater than about 3 tons per acre, and decreases with yield when that is less than about 3 tons per acre.

## 600 *Heterodera schachtii* in Lancashire and Cheshire

The regression lines (a) and (b), represented by the equations

$$A = 0.0737 Y - 4.215 \quad \dots(a),$$

$$Y = 4.75 A + 63.73 \quad \dots(b),$$

have been inserted in Fig. 1. Those equations have been obtained from the coefficients of regression  $b_1 = r\sigma_a/\sigma_y$  and  $b_2 = r\sigma_y/\sigma_a$ , from which  $a = 0.0737 y$  and  $y = 4.75 a$ . The mean values for  $A$  and  $Y$  are respectively 0.73 and 67.2, so that  $a = (A - 0.73)$  and  $y = (Y - 67.2)$ .

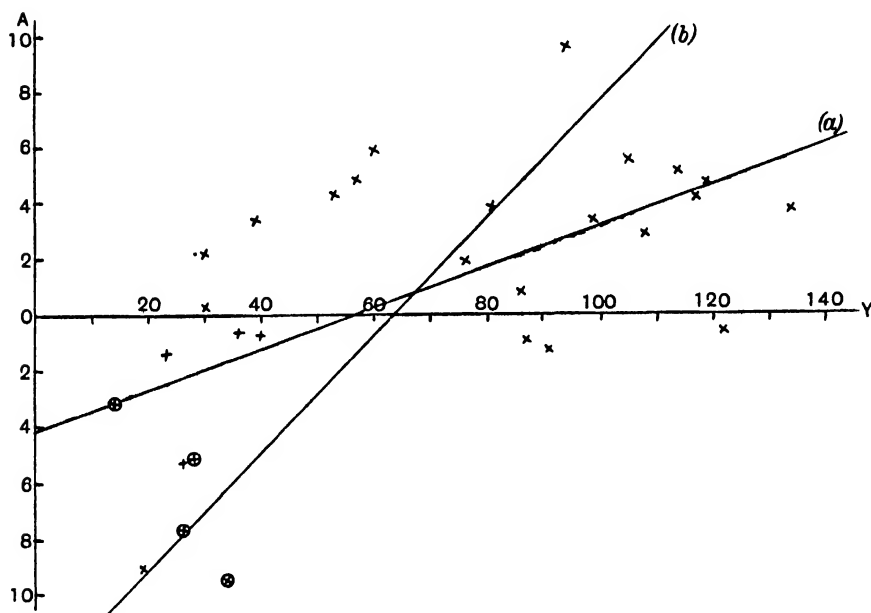


Fig. 1. Diagram showing association between yield ( $Y$ ) and change in cyst content ( $A$ ).

It is evident that the correlation depends upon a number of low values for  $Y$ , four of which are from series 3 and have been encircled. On account of the limitations of the experiments, a close analysis of the plot variation cannot be made, but it is possible that the association between  $A$  and  $Y$  may exist in the variations between plots and be bound up with the unknown factor fertility.

The coefficient of correlation  $r_{AO} = -0.409$  is not affected much by eliminating  $L$  and  $Y$  because  $r_{AO.L} = -0.411$  and  $r_{AO.Y} = -0.414$ . The data, however, do not reveal whether the association between the original concentration and change in concentration of cysts is due to a causal relationship or whether both are affected by another factor. It

has been shown(3), for the soils under consideration, that no apparent association exists between cyst count and physical environment. The observed correlation between *A* and *O* simply indicates that, under the conditions of the investigation, the number of cysts tends to increase on cropping when the original concentration is less than about 17 and *vice versa*. It must be noted, however, that without the four large negative values for *A* from series 3 the observed correlation would become negligible.

#### SUMMARY.

1. Data concerning infestation of *H. schachtii*, certain soil properties and crop yield, from a series of plots at four centres affected with disease, have been collected and examined.

2. Limitations imposed on the design of the plots have prevented a complete analysis of the variates, but the results obtained lead to the conclusion that eelworm infestation is not of primary importance in determining the yield of potatoes and that the cysts tend to increase in number only on a crop which is not a failure but which has been adversely influenced by some other factor.

The authors would like to express their thanks to Messrs I. S. Macdonald, J. Orr, E. Holmes Smith and I. Thomas of the Advisory Department at Manchester for their willing co-operation and assistance, and to Mr L. H. C. Tippetts for his helpful criticism of the statistical treatment of the results.

#### REFERENCES.

- (1) FISHER, R. A. (1928). *Statistical methods for research workers*. 2nd edition. Oliver and Boyd.
- (2) SMITH, A. M. and PRENTICE, E. G. (1929). Investigation on *Heterodera schachtii* in Lancashire and Cheshire. *Ann. App. Biol.* xvi, 324.
- (3) SMITH, A. M. *Ibid.* 340.

(Received April 26th, 1929.)

# POLLINATION OF HARDY FRUITS: INSECT VISITORS TO FRUIT BLOSSOMS

BY G. FOX WILSON

(*Department of Entomology, Royal Horticultural Society's Laboratory,  
Wisley, Surrey.*)

(With 1 Text-figure.)

## CONTENTS.

	PAGE
1. Introduction . . . . .	602
2. Description and plan of orchards in which investigations were conducted .	603
3. Factors influencing fruitfulness in orchards . . . . .	604
4. Anemophilous and entomophilous flowers . . . . .	606
5. Attractiveness of entomophilous flowers . . . . .	607
6. Types of pollination . . . . .	610
7. Pollinating insects and the transmission of pathogenic organisms . . .	610
8. Insects concerned in the pollination of hardy fruit flowers . . . .	611
9. Notes on the chief pollinating agents of hardy fruit flowers . . . .	613
10. Census of chief pollinating agents of hardy fruit at Wisley, Surrey, together with meteorological data (recorded—9 a.m.) of the period during which observations were made . . . . .	623
11. Summary . . . . .	628
References . . . . .	628

## 1. INTRODUCTION.

IN July 1926 the writer published a paper in the *Royal Horticultural Society's Journal* (28) which dealt with the insect visitors to fruit blossoms. That paper was necessarily a popular account on the subject; now a more detailed account of the work as carried out at Wisley during the years 1920–25 is given. The greater number of observations were carried out in the fruit plantations of the R.H.S. Gardens at Wisley, where many hours were spent during the blossoming periods of the various fruits for the purpose of studying the relationship between anthophilous insects and fruit pollination.

The problems were suggested by the Director of Wisley who put forward a plea in 1914(6) to get the matter of the agents concerned in fruit pollination cleared up and suggested that a productive piece of

work would be done if the investigation were taken up seriously. The problems that were set were: firstly, to ascertain the species of insects concerned and, secondly, a study of the habits of the various insects which are found to visit the flowers of hardy fruit.

Whereas previous work in this country on the question of pollinating agents has been confined chiefly to lists of insects found visiting the blossoms (14, 15, 16), detailed observations of their habits have been few and scanty. The mere record of captures at the flowers is of relatively little value, for that takes no note of the number of visits an individual insect may pay.

*Acknowledgments.* I wish to express my sincere thanks to Mr F. J. Chittenden, Director of the R.H.S. Gardens at Wisley, for suggesting the research in 1920 and for reading through this paper and for help and kindly criticism during its progress. Grateful acknowledgment is made of the help received from Mr H. Britten (University of Manchester) and the late Mr E. B. Nevinson (Cobham) in identifying many of the Hymenoptera-Aculeata, and to Mr F. W. Edwards (British Museum, South Kensington) for great help in the identification of the Diptera, and to Mr W. D. Cartwright (Wisley) for allowing me access to the Meteorological Records which are taken daily by him.

## 2. DESCRIPTION AND PLAN OF ORCHARDS IN WHICH INVESTIGATIONS WERE CONDUCTED.

The plantations at Wisley are favoured by reason of their close proximity to open country and pasture land, the district and position of orchards governing to a large extent the numbers of wild insects. There are no grass orchards at Wisley, the land beneath the trees and bushes of all kinds of fruit being cultivated.

The numbers of plants and varieties together with the area which each occupy have been tabulated (Table I), and position of the various fruits are shown (Fig. 1).

Hutson<sup>(17)</sup> remarks that the number of insects other than the hive bee acting as fruit pollenisers in southern New Jersey is small. This is not true of the Wisley plantations, especially in the spring of 1920 when not one hive bee was found on the blossoms of any fruit tree due to the absence of hives within a radius of two miles, and yet an excellent set of fruit was obtained through the agency of wild insects, particularly humble and wild bees.

Table I.

*Type, number and varieties of hardy fruits on which the greater number of observations on their pollinating agents were made.*

Plan figure	Fruit	No. of plants	No. of varieties	Area
1	Almond	2 standards	1	"Sevenacres"
2	Apple	390 bush	189	1½ acre
2 C	Apple	65 cordons	6	130 ft. run
3	Apricot	2 espaliers	1	South wall
*	Cherry	20 half-standards	4	250 sq. yards
4	Cherry	5 espaliers	3	North wall
5	Medlar	1 standard	1	Wild garden
6	Nectarine	5 espaliers	5	South wall
6	Peach	5 espaliers	5	South wall
7	Pear	234 bush	121	¾ acre
7 C	Pear	80 cordons	6	160 ft. run
8	Plum	112 bush	56	½ acre
9	Quince	10 bush	4	120 ft. run
10	Blackberry	30	12 }	320 sq. yards
11	Loganberry	40	1 }	
12	Raspberry	900	32	½ acre
13	Strawberry	540	60	160 sq. yards
14	Black currant	51	8	150 sq. yards
15	Red and white currants	114	6 } 2 }	330 sq. yards
16	Gooseberry	174	72	430 sq. yards
17	Mulberry	1 standard	1	Wild garden
18	Nuts, cob and filbert	45	2	150 yards run
19	Chestnut, sweet	14	1	Wisley Common
20	Walnut	1 standard	1	"Sevenacres"

\* Not shown on plan.

### 3. FACTORS INFLUENCING FRUITFULNESS IN ORCHARDS.

No amount of care to cultural details can induce fruitfulness in a large orchard unless pollen-carrying agents are present in sufficient numbers to ensure adequate cross pollination of the flowers. The lack of pollinating agents may be only one factor influencing fruitfulness in orchards, yet it is too often considered to be a negligible quantity, for less attention has been paid to the essential work of anthophilous insects in the pollination of entomophilous flowers than to any of the under-mentioned factors.

The principal factors governing fruitfulness in orchards are:

(1) Lack of vigour—due to unhealthy root action caused by inefficient drainage and absence of essential soil nutrients or may occur in interplanted bush fruit through dense shade conditions.



(2) Errors in cultivation—a common error is the repeated application of nitrogenous manures which causes an over-production of wood and the failure of the plant to form fruit buds.

(3) Shy-bearing varieties and self-sterile varieties—the massing of self-sterile and inter-sterile varieties and the lack of foresight shown when planting these so that their flowering periods do not overlap.

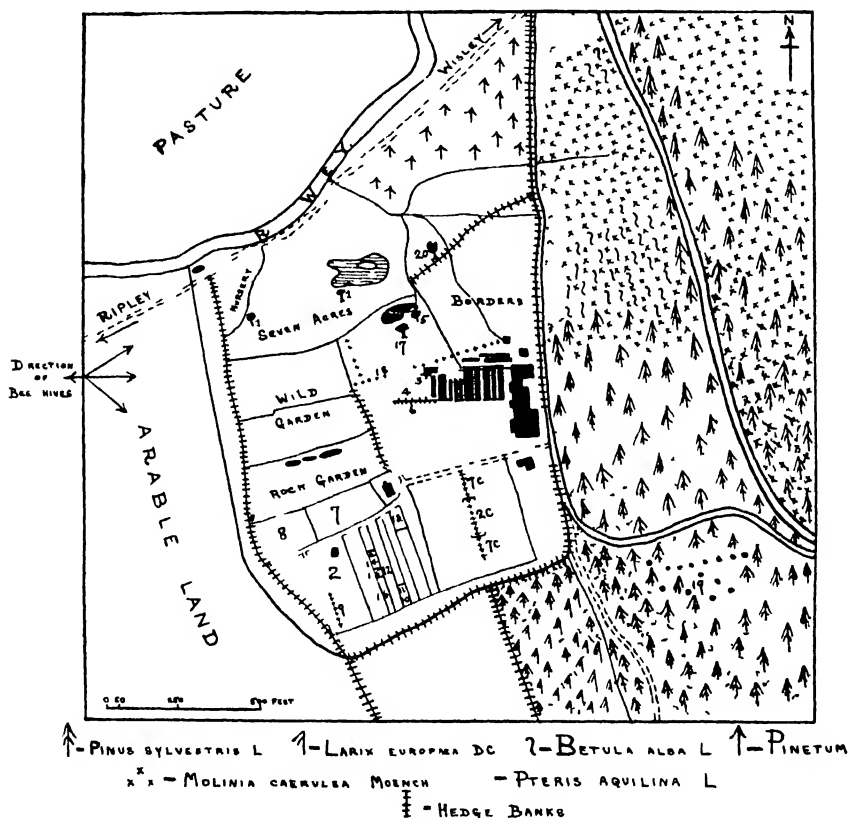


Fig 1. Map of the Wisley Gardens and surrounding district, showing position of the various fruits on which observations were made.

(4) Unfavourable climatic conditions—drying winds injure the receptive stigmas; rain prevents pollen dissemination by closing the anthers or by preventing them from opening (Dorsey (7)); gales and hail showers too often remove the petals and so decrease the attractiveness of rosaceous flowers; low temperatures prevent the setting of fruit,

whilst frost, especially in low-lying regions, kills the stigma and retards the growth of the pollen tube. The indirect effect of unfavourable weather conditions at the time of blossoming is to reduce the number of pollinating agents, particularly domesticated bees.

#### 4. ANEMOPHILOUS AND ENTOMOPHILOUS FLOWERS.

The flowers of our hardy fruits are of two kinds: (1) anemophilous or wind-pollinated, and (2) entomophilous or insect-pollinated. The unfortunate term "fertilisation" is often applied to the transference of pollen from the anthers to the stigma. To ensure fertilisation it is usually necessary that the pollen and stigma shall be of the same species of plant, and this transference through the agency of wind or insects is termed pollination.

*Anemophilous flowers.* Among our hardy fruits, anemophily occurs in three natural orders—*Urticaceae* (mulberry), *Cupuliferae* (nuts and chestnut) and *Juglandae* (walnut).

Insects are rarely attracted to these obscurely coloured flowers, but records show that hive bees will occasionally visit the male flowers of nuts and walnut for the purpose of collecting pollen. The male catkins of chestnut attract a limited number of flies by reason of the aminoid odour, to which certain flies are positively chemotropic.

The researches of Chittenden (5) in England and Lewis and Vincent (19) in America clearly show that there is no ground for supposing that the pollen of apple, pear, plum and cherry is carried by wind. The method adopted was to place vaseline- or glycerine-smearred glass plates at different heights amongst plantations of apple and plum and at varying distances from the trees. At Wisley, the only apple and plum pollen grains collected were found near the remains of insects that had alighted on the plates. Pine pollen was caught in great quantity, the nearest pine trees being about a quarter of a mile away. The American workers only found 67 apple pollen grains on 20 plates. Backhouse (1) carried out some experiments in an English orchard to discover whether wind is capable of pollinating the flowers of a self-fertile variety of plum ("Victoria"). By covering the flowers on one-third of the tree with muslin to exclude insects and yet allow for currents of air to have free play among the blossoms, only one fruit set on the covered portion, whereas a heavy crop resulted on the uncovered portion.

*Entomophilous flowers.* Among the 16 fruits with entomophilous flowers, 13 belong to the natural order *Rosaceae* and 3 to the *Saxifrageae*.

To consider briefly the several entomophilous fruit flowers, the almond is the first to flower, but the weather is seldom favourable for visits from pollinating insects. Amongst apple and pears, we find none sufficiently self-fertile to be planted in large blocks and still to give good yields. Morello cherries and certain varieties of plums (Yellow Pershore, Egg and Victoria), unlike other varieties of cherry and plum, are self-fertile to such a degree that they may be planted in blocks without interspersing other varieties. The flowers of peach and nectarine, when grown on outside walls, receive very few visits from insects, and it is necessary to pollinate the flowers by hand. Blackberry, loganberry and raspberry flowers are sought after chiefly for pollen, which is collected in large quantities by hive, humble and wild bees and, although self-fertile, better and more perfect fruit is produced when pollinating insects have access to the flowers. Raspberry flowers are less favoured than those of blackberry and loganberry by insect visits. The strawberry is less dependent on visits from insects than any other fruit flower and is partially pollinated by wind. Many American varieties are dioecious, and care has to be taken to see that pistillate forms do not predominate. Black, red and white currants and gooseberry depend entirely on the visits of insects for the transference of the glutinous spherical pollen grains. To exclude insects from the flowers of these plants is to court disaster (Reid<sup>(25)</sup>), and the practice of fixing a cage made of fine mesh netting over the plantations is to be deprecated. One result of imperfect pollination in black currants is to cause "running-off" (Hatton<sup>(13)</sup>), that is where only a few flowers on each truss set fruit. The trouble occurs in large plantations when there are an insufficient number of pollinating agents and in small plantations when covered with small mesh netting so that insects are excluded. The introduction of hives of bees reduces the danger.

##### 5. ATTRACTIVENESS OF ENTOMOPHILOUS FLOWERS.

The interdependence of flowers and insects cannot be disputed, and entomophilous flowers depend on their colour and fragrance to attract pollinating insects when the pollen is ripe and the stigma is receptive. The attractiveness of colour may often be a difficult factor to explain, for the human appreciation of colour may be different from that of insects. Recent work on this question has been carried out by Lutz<sup>(21)</sup>, who points out that insects are noted for poor vision and a strongly developed sense of smell. Anthophilous insects are known to visit in

large numbers plants with small and inconspicuous flowers, as witness the numbers of wild, humble and hive bees to the flowers of currants and gooseberry. To such flowers they are attracted by a sense of smell. Lutz has shown that pollinating insects can see ultra-violet as well as, or even better, than they can see the rays perceived as light by man. Observations based on about one hundred flowers show that most of the "yellow," many "red" and "blue" flowers are strongly ultra-violet, but that few or no "white" flowers are so.

In order to test how far the floral envelope plays a part in attracting insects, series of experiments have been made in America and England. Lewis and Vincent<sup>(19)</sup> found that although flowers deprived of their petals were much less attractive, yet several insects pay some visits to blossoms from which the corollas have been removed. Lovell<sup>(20)</sup>, working with pear blossoms, found that once the flowers were deprived of their petals honey bees ceased to visit them for nectar. His two series of experiments may be briefly summarised as follows:

Seven flowers watched for 15 minutes received 8 visits from hive bees. The same 7 flowers deprived of their petals when watched for 15 minutes received no visits and, when watched for a further 15 minutes received 2 visits from hive bees due in part to association (*sic*). Again, one cluster comprising 8 blossoms with petals was watched for 15 minutes and it received 11 visits from hive bees; the other cluster of 8 flowers with the petals removed received no visits.

The author's conclusions are that the bees were guided almost entirely by the presence of petals. That the Wisley experiments do not confirm these results may be seen by consulting Table II.

Observations were carried out on 4 cordon apples, variety "Ecklinville Seedling," two of which had the floral envelope removed from open and unopen flowers on April 29th, 1921, the two remaining cordons being left as controls.

The results as to the insect visitors to normal flowers and to flowers from which the petals were removed are based on observations carried out from April 23rd–May 5th, 1921, over a period of 4 hours 5 minutes at various times of the days in question, generally between 10 a.m. and 6 p.m.

It was found that apple flowers denuded of their petals depended mainly on hive bees, less so on species of *Syrphus* and Anthomyiids, for pollination. Humble bees passed over trees devoid of petals and preferred normal trees situated between and on each side of the abnormal ones. The theory has been put forward that the greater number of hive

Table II.

*Insects visiting apple flowers with and without petals.*

Species	No. of insects		No. of flowers visited	
	Normal flowers	Denuded flowers	Normal flowers	Denuded flowers
HYMENOPTERA				
<i>Apis mellifica</i> ...	84	23	1183	432
<i>Bombus agrorum</i> ...	16	1	152	37
<i>B. lapidarius</i> ...	2	0	9	0
<i>B. lucorum</i> ...	10	1	88	1
<i>B. terrestris</i> ...	5	0	76	0
<i>Andrena fulva</i> ...	4	3	29	15
<i>A. nana</i> ...	2	1	15	2
DIPTERA				
<i>Sciarinae</i> ...	—	Few	Not counted	
<i>Bibio marci</i> ...	1	0	3	0
<i>Syrphus torvus</i> ...	25	5	123	11
<i>S. balteatus</i> ...	21	6	89	36
<i>S. ribesii</i> ...	1	2	7	13
<i>Eristalis tenax</i> ...	4	0	11	0
<i>Bombylius major</i> ...	1	0	1	0
<i>Calliphora erythrocephala</i> ...	3	1	4	2
<i>Anthomyiinae</i> ...	Many	Many	Not counted	
COLEOPTERA				
<i>Phyllopertha horticola</i> ...	1	0	1	0
<i>Adalia bipunctata</i> ...	2	1	6	2
LEPIDOPTERA				
<i>Pieris napi</i> , ♂ ...	1	0	1	0
Total ...	183	44	1798	551

bees over other insects on the abnormal flowers is due to the more diligent and systematic search which these insects make when seeking food. The result of another experiment may show that this is not the sole explanation. Artificial apple flowers, anatomically correct, were placed among clusters of apple flowers in one of our orchards, but hive bees were not attracted to them until nectar was placed at the base of the linen petals, after which the bees visited them for the nectar and were attracted to them by the scent of the nectar. The attractive force was odour and not colour. The olfactory sense of humble bees is less keen than the visual and plays a smaller part in the physiology of the insect than in the case of the hive bee. This appreciation for the presence of the floral envelope is shown in Table II, where the numbers of humble bees that visited abnormal flowers are negligible. On the other hand, several individuals

(*Bombus lucorum* and *B. terrestris*) were seen to visit artificial flowers without nectar, the presence of which was necessary before hive bees could be induced to settle on them. *B. lucorum* was found to alight on fallen petals lying beneath apple and plum trees, a fact which shows the important part which the floral envelope plays in attracting these insects.

#### 6. TYPES OF POLLINATION.

The pollination of hardy fruits grown under natural conditions takes place through the agency of wind (anemophily) and anthophilous insects (entomophily).

Peaches and nectarines are usually grown under glass, whilst many varieties of apple, pear, plum and cherry are grown as pot fruit and partially forced under glass. Pollination under these circumstances must be carried out artificially by hand when either a camel-hair brush for experimental work or a hare or rabbit's tail for general purposes is used. Some growers introduce a hive of bees into fruit houses during the blossoming period with complete success so far as pollination is concerned, but the effect on the bees is injurious as will be explained later.

#### 7. POLLINATING INSECTS AND THE TRANSMISSION OF PATHOGENIC ORGANISMS.

That there is real danger arising from the visits of insects to fruit flowers is certain and bees and other anthophilous insects have at various times been shown to transmit pathogenic organisms from flower to flower. Barker and Grove<sup>(2)</sup> have described a bacterial disease of pear blossom, and the conclusions reached were that infection is spread from infected to healthy blossoms through the agency of bees. It was found that healthy flowers were inoculated through the stigma or parts of the flower with which the feet of the insects came into contact. Several bees, which had visited infected flowers, were transferred to sterile Petri dishes and in 50 per cent. of the cases colonies of this particular bacterium were found in the footprints after an interval of three to four days. Bond<sup>(3)</sup> has observed that whereas the pollen of unopened flowers and those of anemophilous flowers are generally free from microbial infection, that obtained from flowers frequented by hive bees, various species of wild bees and other insects was not. Spore-bearing, gram-negative bacilli together with other bacillary and, in some cases, coccal forms were frequently grown from open entomophilous flowers. It seems probable

that many kinds of open flowers frequented by bees and other insects harbour enormous numbers of organisms, some of which are pathogenic to bees under certain conditions, and the writer suggests that further study of the bacterial flora of flowers would shed light on the diseases to which bees and other insects, besides other animals and even man, are heir. Doidge<sup>(6)</sup> in her researches on a bacterial blight of pear in South Africa due to *Bacterium nectarophilum* found that the disease organisms were transmitted by bees and other insects. Blossoms covered with paper bags gave a 100 per cent. set, whereas a large percentage of uncovered flowers wilted and fell off. This was attributed to the sheltering effect from high winds and sudden changes in temperature, but it is suggested that it is due mainly to the exclusion of anthophilous insects. Cultures of the causal organism were obtained from bee traces across sterile media in tubes. Other cultures were obtained when the head and thorax of a bee were dropped into sterile plates. Ants are suspected of being concerned in the transmission of the disease.

The case of mite transmission may here be cited. Great numbers of the black currant mite, *Eriophyes ribis* Nal., are found on the foliage and flowers of currants during the migration period, and we have discovered isolated specimens on the legs of *Bombus species* captured whilst visiting the flowers of mite-infested bushes. The danger from the dispersal of mites clinging to the bodies of anthophilous insects must be considered as an ever present menace to mite-free plantations.

#### 8. INSECTS CONCERNED IN THE POLLINATION OF HARDY FRUIT FLOWERS.

No attempt has been made to arrange the orders, families and genera of plants and insects in strict genealogical order. The families of insects are arranged in accordance with the importance of their members as pollinating agents, whilst the species are in alphabetical order for clarity.

Müller<sup>(23, 24)</sup> and Knuth<sup>(18)</sup> give lists of insects visiting the flowers of 14 and 9 different kinds of fruit flowers respectively. Walton<sup>(27)</sup> lists the number of *Bombus species* found visiting fruit flowers in North Wales. Hatton<sup>(13)</sup> records the insects taken on black currant flowers at East Mallang.

## Number of species of insects taken on fruit flowers at Wisley.

	HYMENOPTERA					DIPTERA			COLEOPTERA		HEMIPTERA					
	Apidae	Bombidae	Megachilidae	Andrenidae	Others	Syrphidae	Muscidae	Others	Beetles	Weevils	LEPIDOPTERA	NEUROPTERA	Heteroptera	Homoptera	THYSANOPTERA	ORTHOPTERA
Apple ...	2	5	1	9	8	14	6	17	15	4	4	2	1	2	1	—
Pear ...	2	3	1	6	4	6	5	6	8	2	—	—	—	—	1	—
Quince ...	2	4	—	3	1	2	2	2	4	3	1	—	—	—	—	—
Medlar ...	1	1	—	—	—	1	—	—	—	—	—	—	—	—	—	—
Cherry ...	2	5	1	4	2	6	2	4	2	1	1	—	—	—	—	—
Almond ...	—	2	—	—	—	1	—	—	—	—	—	—	—	—	—	—
Apricot ...	—	—	1	1	—	—	—	—	—	—	—	—	—	—	—	—
Peach and nectarine }	1	2	—	—	—	1	1	—	—	—	—	—	—	—	—	—
Plum ...	2	3	1	8	3	7	3	3	4	—	2	—	—	1	—	1
Blackberry ...	2	4	—	3	1	9	1	4	3	—	1	—	—	—	—	1
Loganberry ...	2	4	—	4	1	11	1	2	4	—	—	—	1	—	—	—
Raspberry ...	2	7	—	9	1	6	—	2	3	—	2	—	1	1	—	—
Strawberry ...	2	3	—	2	1	3	1	2	2	—	1	—	—	—	—	—
Black currant ...	2	4	—	2	3	2	1	4	1	1	1	—	—	—	—	—
Red and white currants }	2	3	1	5	5	4	1	7	7	—	1	—	1	1	—	—
Gooseberry ...	2	4	—	3	4	2	1	3	4	—	1	—	—	1	—	—
Mulberry ...	No records of insect visits															
Nuts, cob and filbert }	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Chestnut ...	1	—	—	3	1	7	6	9	3	—	—	—	1	—	—	—
Walnut ...	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

## Local larval habitats of chief pollinating insects of hardy fruit flowers.

## HYMENOPTERA

Apidae	<i>Apis</i> species	In hives; occasionally in hollow trees (Wisley Common)
Bombidae	<i>Bombus</i> species	In hedge banks and headlands amongst grass tufts and moss (pinetum and Common)
Megachilidae	<i>Osmia rufa</i>	In ground, old wooden fences and palings, gateposts, old bamboo canes, keyholes and snail shells (in and around R.H.S. Gardens)
Andrenidae	<i>Andrena</i> , <i>Halictus</i> species	In hedge banks, sloping grass banks amongst grass tufts, in gravel paths, in hollow blackberry stems (in and around Gardens and Common)
Vespidæ	<i>Vespa</i> species	In the ground or suspended from trees and shrubs (in and around Gardens)



*Local larval habitats of chief pollinating insects of hardy fruit flowers*  
(continued).

## DIPTERA

Mycetophilidae	<i>Sciara</i> species	In decaying vegetable refuse and beneath cow-dung and decayed bark of trees (in pastures and Gardens)
Bibionidae	<i>Bibio marci</i> , <i>Dilophus febrilis</i>	In living and decaying vegetable matter (in and around Gardens)
Bombyliidae	<i>Bombylius major</i>	Parasitic upon species of <i>Andrena</i> and <i>Halictus</i> (which see)
Syrphidae	<i>Syrphus</i> species	Predaceous upon various species of Aphides on ornamental flowering shrubs, fruit trees and vegetables (Gardens)
	<i>Platychirus albimanus</i>	In decaying organic matter in damp situations (in Gardens and on Common)
	<i>Rhinga rostrata</i>	In cow-manure (pastures)
	<i>Volucella</i> species	Scavengers in nests of <i>Bombus</i> and <i>Vespa</i> (which see)
	<i>Eristalis</i> species	In seepage water from pigsties and liquid manure tanks (Gardens)
	<i>Merodon equestris</i>	In narcissus bulbs
Cordyluridae	<i>Scatophaga stercoraria</i>	In cow-dung (pastures)
Anthomyiidae	Anthomyiids	In living and decaying vegetable matter and manure (Gardens)
Muscidae	<i>Calliphora erythrocephala</i> , <i>Lucilia caesar</i>	In decaying animal and vegetable matter (Gardens and Common)
COLEOPTERA		
Nitidulidae	<i>Meligethes aeneus</i>	Phytophagous on certain <i>Cruciferae</i> (head-lands and waste land round Gardens)
Coccinellidae	<i>Adalia bipunctata</i> , <i>Coccinella 7-punctata</i>	Predaceous upon various species of aphides on ornamental flowering shrubs, fruit trees and vegetables (Gardens)

## 9. NOTES ON THE CHIEF POLLINATING AGENTS OF HARDY FRUIT FLOWERS.

The various orders and families are considered in relation to the importance of their members as pollinating agents.

## Order HYMENOPTERA.

Fam. **Apidae**. By their more diligent habits, numbers, perceptive qualities and body structure, hive bees play the most important part in the pollination of flowers. They are a governable quantity and are essential for carrying out the work of pollination in large orchards and plantations where it is rarely possible to depend on the work of wild insects. The presence of hive bees in all fruit plantations, especially where large areas are devoted to one kind of fruit, makes cross-pollination doubly sure.

Unfavourable climatic conditions reduce flight, and a long series of observations carried out at Wisley and elsewhere shows that hive bees are affected by low temperatures, even when accompanied by bright sunshine, high percentage of relative humidity in the atmosphere, rain, sleet and snow showers, the presence of cloud, absence of sunshine and high winds (that is above 10 m.p.h.) and cold northerly and easterly winds. The indirect effect of these factors is reduced nectar secretion.

Hive bees work diligently and systematically during bright, sunny, warm weather and will travel to a distance of at least two miles in search of rosaceous flowers. For at least two years, prior to 1921, the hive bees in the neighbourhood of the Wisley fruit plantations were exterminated by disease, yet the crops of apples, pears, plums, currants and gooseberries in 1919 and 1920 were above the average, this being entirely due to the work of humble and other wild bees and flies.

A strong colony of hive bees commences flight at a lower temperature than a weak one, and it is essential for fruitgrowers to obtain females or "swarm" from a healthy stock. Recent work carried out in New Jersey(17) shows that it is necessary to allow one hive for an acre of fruit.

It is not unusual to find hives placed in peach houses in early spring, but this practice is to be deplored for the early activity cannot be maintained, there being an insufficient number of flowers in the open, whilst great numbers of bees die in attempting to escape from the house. The difference in temperature between glasshouse and open air at this period of the year is too great, and the result will be a weakened stock and a great loss in individuals.

During the first warm days in February and March hive bees become active and leave the hive in search of food in the form of pollen and nectar. We have records of bees visiting the male catkins of cob and filbert nuts for pollen.

It has previously been shown that the olfactory sense of honey bees is strongly developed and possibly more so than the visual. This statement whilst agreeing with the conclusions reached by Frisch(10, 11, 12) and Plateau is at variance with those of Wery, who found that bees were as readily attracted to flowers which were completely enclosed in glass globes as those exposed. The observations of Frisch show that when a rich supply of nectar is discovered by a bee, it returns to the hive and executes a series of gyratory movements. Other bees come in contact with it and stroke the abdomen of the dancing bee with their antennae. The bees then leave the hive and search in ever-widening circles for the source of nectar; the number of bees attracted being proportional to the supply. The attractiveness is due to both the scent of the flower and the odour of a volatile substance secreted from a scent-gland situated on the abdomen of the bee and which it has left on the flower. The attraction towards a source of pollen supply is as great, but the character of the "dance" is said to be different from that performed by the nectar collectors.

The number of flowers visited by hive bees in one minute depends on the type of flower (accessibility of nectar and pollen), its condition (partially or fully open) and climatic conditions. During bright, warm sunny weather the rate of visits is accelerated and the average number of apple, pear, plum and cherry flowers visited was 9.6, whilst those of currants works out at 8. The presence of cloud and slight wind slowed the visits down to 6.5 and 5 respectively, and as the weather became stormier their visits ceased altogether.

Two species have been taken on all entomophilous fruit flowers at Wisley since 1921. The common honey bee, *Apis mellifica*, and the Ligurian bee, *A. ligustica*, were frequent visitors to rosaceous flowers and less so to currants and gooseberry, although these flowers depend on visits from anthophilous insects for the transference of the globular and glutinous pollen.

Great danger attends the application of arsenical washes to fruit trees during the period of blossoming. The spraying campaign in orchards must be arranged to

avoid the use of poisonous sprays, particularly those containing arsenic and nicotine, during the flowering period. Attention to this malpractice has been made repeatedly by the Ministry of Agriculture, the South-eastern Agricultural College at Wye and the Royal Horticultural Society through the medium of the horticultural press. The effect on hive bees of spraying fruit trees in blossom with arsenicals has been demonstrated by McIndoo and Demuth(22). It was ascertained that bees work equally well on trees sprayed in full flower as on unsprayed ones, and that they do not fly away from the sprayed orchards to any marked degree if the orchard is well isolated, but they are slightly affected when a small orchard is sprayed in full bloom. Orchard routine may include the application of Bordeaux-lead arsenate spray during the "pink" stage and, as this stage is often of short duration, spraying is continued when many of the blossoms have burst open. It was established by these workers that the minimum fatal dose of arsenic per bee is 0.0004–0.0005 mg.

**Fam. Bombidae.** Nine species of *Bombus* and one species of *Psithyrus*, one of the "cuckoo" bees, have been taken on fruit blossoms. The plantations at Wisley are favoured by their close proximity to open country and pasture, in which humble bees make their nests without fear of disturbance. Where fruit plantations are situated in areas surrounded by building land, such as may be found in suburban areas, and in districts where hedges, with their attendant headlands, are replaced by fences and clean-cut ditches, the number of humble and other wild bees is limited to a number insufficient for the carrying out of pollination.

Fruit flowers belonging to the *Rosaceae* and the *Saxifrageae* were favoured by four species of humble bees, viz. *B. pratorum*, *B. lapidarius*, *B. lucorum* and *B. terrestris*. The last two species are similar in their times of appearance and general activities as witness the number of flowers visited in one minute by each:

(i) <i>B. lucorum</i>	16	apple	(ii) <i>B. terrestris</i>	18	apple
	16	pear		16	pear
	16	plum		15.6	plum
	14.6	cherry		13	cherry
	12	gooseberry		12	gooseberry

Humble bees, unlike hive bees, are not deterred from their pollinating activities during inclement weather. Observations carried out over a period of five years(28) show that many species of *Bombus* continue to visit various fruit blossoms during (i) high winds and gales, (ii) cold winds, (iii) heavy and continual rain, (iv) snow and hail showers, (v) dull and overcast weather and (vi) from early morning to late evening. During wet and windy weather they are less active and crawl from flower to flower with greater deliberation than during bright sunny weather.

*B. pratorum* puts in an early appearance during March and April and aestivates in July prior to hibernation. It is a diligent worker, the number of flowers visited in one minute being higher by 1.5–2 than with *B. lucorum*. *B. agrorum* confines itself to the blossoms of apple, cherry, quince and gooseberry flowers, whilst *B. helveranus*, *B. jonellus* and *B. muscorum* have only been taken on raspberry flowers. *B. ruderatus* was occasionally seen on black currant. *B. helveranus* is the last to emerge and does not make an appearance until the end of June and continues throughout July.

Walton(26,27) remarks that *B. lapidarius* is a late appearing species and is not a frequent visitor to fruit blossoms with the exception of apple and blackberry, but

we have found it visiting the flowers of eight rosaceous plants and also black currant. It is rather an erratic worker and chooses to alight on the uppermost branches of apple, plum, pear and cherry trees and from there working downwards to the lower branches.

Humble bees are more attracted to apple and plum flowers than to pear, for the hawthorn odour of these blossoms, due to trimethylamine, proves somewhat distasteful to them.

The first bees to be observed on early flowering varieties of fruit trees are all females, the neuters appearing later in time to carry on the work of pollination of late flowering varieties of apple, pear, plum and cherry.

*Psithyrus quadricolor* is associated with *B. pratorum* and *B. jonellus* and was an occasional visitor to the flowers of blackberry and loganberry. Species of "cuckoo-bees" do not possess the pollen-carrying apparatus on the posterior legs, and their visits to flowers are entirely for selfish reasons, while their unmethodical habits make them useless from a pollination standpoint.

**Fam. Megachilidae.** *Osmia rufa*, one of the mason bees, is found abundantly in most fruit-growing areas. Its movements, though slow, do not detract from its importance as a useful agent in the pollination of many fruit flowers. It is a diligent worker, its chief characteristic is its habit of alighting in the middle of rosaceous flowers and crawling over and round the anthers and style. Its nest is formed in hollows in the ground, in old wooden fences and palings, gateposts and keyholes, and even in snail shells.

**Fam. Andrenidae.** This family is an extensive one and 17 species of *Andrena*, 5 species of *Halictus* and 1 species of *Sphcodes* have been taken on fruit flowers. Members of this family, though known as solitary bees, are often gregarious in their habits, for they choose to make their nests together in a small area and form colonies. They are great pollen collectors, with the exception of species of *Sphcodes* which possess only rudimentary pollen-carrying apparatus.

The species of *Andrena* and *Halictus* vary to a great extent in size, this being found both individually and specifically. Many species are double-brooded, the first brood occurring in April and May and the second in July and August, and one finds the broods often differ slightly in appearance, especially among the males.

Their presence in orchards during the period of blossoming, though desirable, cannot be ensured with the same ease as with hive bees. Most species construct burrows in vertical hedge banks, sloping banks, among grass in headlands and waste land and in gravel paths. The proximity of the orchard to common land and undisturbed areas ensures the presence of many species of this family.

Among the species of *Andrena* that visit fruit flowers, a curious habit has developed, more especially in the spring broods, in the times of their appearance on the blossoms. In the number found and in the increased activity shown, they are strongly represented during the hours from 11 a.m. to 1 p.m. (standard time). Between 1 and 2 p.m. they are torpid and often disappear altogether, but reappear again with lessened activity from 2 to 4 p.m. They prefer to work in flowers during bright sunny weather, and their activities are immediately lessened during the temporary disappearance of the sun behind clouds, at which time they may be found resting in the blossoms or on the ground, resuming their labours on the reappearance of the sun. Very few are found during showery weather, when they either do not venture far from their colonies or crawl sluggishly among the blossoms.

*A. albicans* and *A. fulva* were most frequently met with on the flowers of hardy fruits, visiting 10 and 7 species respectively. The greatest number of species were taken on apple flowers, whilst raspberry, plum and currants were favoured in their respective order.

The number of individuals in *Halictus* was small, the favourite flowers being blackberry, loganberry and raspberry.

Among the "Sphecode" bees, *S. gibbus* was the only species taken, and confined its attention to apple, plum and cherry flowers.

Fam. **Nomadidae**. Members of this family live asinquilines in the nests of wild bees and are associated more especially with *Andrena* species. The two species taken, one on raspberry and the other on red currant flowers, were uncommon and of little importance as pollinating agents.

Fam. **Eumenidae**. *Odynerus callosus* is the sole species of Solitary True Wasp that has been taken on fruit flowers (apple, pear and plum) at Wisley. Although the females collect small lepidopterous larvae for the purpose of storing their nests as food for the larvae, they occasionally visit flowers and feed on nectar and pollen.

Fam. **Vespidae**. Two species of ground-nesting social wasps, *Vespa rufa* and *V. vulgaris*, and one species of aerial-nesting wasp, *V. sylvestris*, were occasionally seen to visit fruit blossoms during early spring. The females seek nectar and pollen and, on occasion, became predaceous on aphides and lepidopterous larvae that are crawling over the flower clusters. *V. vulgaris*, ♀, was once observed licking the nectar from the leaf nectaries on cherry.

Fam. **Formicidae**. Ants, although frequent visitors to flowers for the purpose of licking nectar, are not useful pollinating agents because they carry but a small amount of pollen on their smooth bodies and do not pass from tree to tree. They are great marauders of unopened pear blossoms, which they frequently bite through in order to reach the nectar.

Fam. **Tenthredinidae**. Sawflies, by reason of the phytophagous habits of their larvae, are unwanted visitors in orchards. The apple sawfly, *Hoplocampa testudinea*, was abundant during the flowering season of 1921, 1922 and 1925. The ♀♀ choose the open flowers for the deposition of their eggs. The insects are extremely active during sunny days and flit from flower to flower with great rapidity.

The gooseberry sawfly, *Pteronus ribesii*, was an occasional visitor to the flowers of currants and gooseberry for nectar, but, unlike the last-mentioned species, the eggs are deposited on the leaves.

#### Order DIPTERA.

Fam. **Cecidomyiidae**. A family comprising the gall midges, the presence of which is as undesirable as those of the last family. The raspberry stem gall midge, *Lasioptera rubi*, was a frequenter of apple flowers in 1922. The only other species taken on fruit blossoms was the pear midge, *Contarinia pyrioxora*, an ever present pest in many pear orchards but variable in numbers from one year to another.

Fam. **Mycetophilidae**. Members of the family of fungus gnats are classed under the general term "*Sciara species*" in the lists of insects taken on fruit flowers. In numbers they surpass all other insect visitors to apple, pear, plum, cherry, quince, strawberry, currant and gooseberry flowers. Owing to their small size their effectiveness as pollen carriers is overlooked, but the amount of pollen distributed by them

must be considerable. They may be found flitting from flower to flower at all times of the day and during stormy weather when very few insects venture far from their nests.

**Fam. Chironomidae.** Two species of *Chironomus* were taken on apple blossom, but their visits are less purposeful than any other pollinating insect. One species each of *Orthocladus* and *Trichocladus*, however, were not uncommon on the flowers of apple, their habits resembling those of species of *Sciara*.

**Fam. Bibionidae.** The St Mark's fly, *Bibio marci*, was a frequenter of apple flowers and may be classed as a useful pollinating agent.

The most abundant species was *Dilophus febrilis*, which was numerous on apple, pear and cherry. Edwards(9) says of this species that it is "probably one of the most important agents in the fertilisation of fruit blossom."

**Fam. Bombyliidae.** *Bombylius major* was an occasional visitor to apple blossom and is strongly heliotropic, for it has never been seen on dull days and ceases its visits during cloudy spells. The trophi are adapted for sucking nectar from tubular flowers such as primrose. The parasitic larvae, according to Chapman(4), are found in the cells of *Andrena labialis* and other species of *Andrena* and *Halictus*.

**Fam. Empididae.** *Empis* species are not truly anthophilous, but may be seen on occasions to suck the nectar of fruit blossom. *E. opaca* and *E. tessellata* were frequent visitors to apple flowers. Their habits are erratic which minimises their usefulness as pollinators.

**Fam. Syrphidae.** This family is strongly represented, many species being found on fruit blossom for the purpose of sucking nectar and feeding on pollen. The larvae may be classified as (i) entomophagous (*Syrphus*), (ii) saprophagous (*Eristalis*, *Platychirus*, *Rhingia* and *Volucella*) and (iii) phytophagous (*Merodon*).

*Platychirus albimanus* is the smallest Syrphid acting as a pollinator to fruit blossom, and by reason of its numbers may be classified as a useful agent.

Ten species of *Syrphus* are recorded as being attendants on fruit blossom, the most abundant being *S. ribesii* and *S. torvus*, visiting 5 and 11 different species of fruits respectively. Their work is somewhat spasmodic, for they frequently return again and again to the same blossom, after hovering in the air above the flower clusters. As many as 45 flowers are visited in one minute, and their movements from one tree to another are carried out with great rapidity. *S. torvus* is a slower worker and, consequently, a more useful pollinating agent. Apple, blackberry and loganberry flowers attracted great numbers of Syrphids. Three species were frequently found on flowers denuded of their petals (Table II), the odour of nectar proved to be the attractive force. Artificial flowers were not attractive unless nectar was present on them.

*Rhingia rostrata* is an industrious and frequent worker of apple, pear and blackberry flowers, and *R. campestris* confined itself to cherry blossom. They are fine-weather workers and prefer hot sunny days.

Two species of *Volucella*, *V. bombylans* and *V. pellucens*, were taken on rosaceous flowers. The larvae of the former species are scavengers in the nests of *Bombus*, the latter in the nests of *Vespa*. Their work among fruit blossom is casual and they cannot be classed as useful agents.

The genus *Eristalis* was represented by 5 species, of which 3, viz. *E. arbustorum*, *E. pertinax* and *E. tenax*, were constant attendants on fruit flowers. Their food

consists of nectar and pollen, which they suck up through the proboscis. The excretum of these insects is frequently seen to consist entirely of disintegrated pollen grains. Their habits resemble those of certain species of *Syrphus*, for they work spasmodically and spend a great deal of time hovering over the flower clusters. The resemblance of *E. pertinax* and *E. tenax* to hive bees is marked, both in the way in which they settle on flowers and in the position taken up by the posterior legs when the insects are in flight. Both species have been taken on the flowers of ten species of fruit. *E. arbustorum* stands out as being a more diligent worker. *E. intricarius* visits a limited number of rosaceous flowers and is far less regular in its habits, whilst *E. nemorum* was only taken on blackberry and loganberry flowers. The rat-tailed larvae of these insects are common inhabitants of seepage water from pigsties and liquid manure tanks at Wisley.

The narcissus fly, *Merodon equestris*, confines its attention to apple blossom. It is very agile on the wing and flits from flower to flower, visiting only two or three blossoms on each tree during bright, sunny days.

Fam. **Conopidae**. *Myopa polystigma* was an occasional visitor to apple and cherry flowers. Their habits are sluggish, and they allow themselves to be picked off the blossoms. The larvae are endoparasites of adult bees (*Andrena* and *Bombus*) and wasps (*Vespa*).

Fam. **Cordyluridae**. The common cow-dung fly, *Scatophaga stercoraria*, which is a frequenter of meadows where the eggs are deposited in cow-dung, is found abundantly on fruit blossoms. It resembles *Eristalis* in being partial to the fishy-scented flowers of pear. The tawny-coloured male far outnumbers the dingier-looking female.

Fam. **Anthomyidae**. This very extensive family was represented by several species, but they have not been determined. They are present in large numbers on all fruit blossoms, being partial to rosaceous flowers, from which they can readily obtain nectar. They resemble *Sciara* in that their visits are less purposeful, but their work cannot be ignored for, although small in size, they are indefatigable workers under both calm and stormy conditions.

Fam. **Muscidae**. *Calliphora erythrocephala* may be classed as an important agent in the pollination of apple, pear and plum flowers. Bluebottles frequent these flowers in great abundance, and have been taken on the blossoms of ten other species of fruits. They suck up nectar and are not averse to sucking up rain- and dew-drops on the petals and foliage. Their work is somewhat unstable for they fly away as soon as the tree is approached, but their importance lies in that they are numerous and that they work amongst the blossoms during inclement weather.

*Lucilia caesar* is less abundant and its erratic habits make it comparatively useless as a pollinating agent.

*Musca autumnalis* De G. (*corvina* F.) is a common attendant on apple, pear, plum and cherry flowers which it frequents during fine and rainy weather. Its usefulness cannot be questioned.

Fam. **Sarcophagidae**. We do not expect to find anthophilous habits among the pupiparous flesh flies, but *Sarcophaga carnaria* may be frequently seen on the flowers of blackberry, loganberry and raspberry. It is a somewhat heavy insect in the way in which it alights on the blossoms. Its hairy legs become covered with pollen grains as it walks over and around the stamens in its search for nectar.

●

Order COLEOPTERA.

The bodies of most beetles which are found in flowers are devoid of pubescence, and the amount of pollen which they carry from flower to flower is usually negligible. The habit of remaining in one flower for a considerable length of time does not allow them to be classed as useful agents in the pollination of fruit trees.

**Fam. Byturidae.** The raspberry beetle, *Byturus tomentosus*, is a serious pest of raspberry, loganberry and cultivated blackberry, and is an unwanted addition to the already large coleopterous fauna of flowers. The females, besides depositing their ova in the flowers, feed on the floral organs and, occasionally, bite through the stalk so that the blossoms drop off.

**Fam. Coccinellidae.** Aphidivorous ladybirds, of which four species have been taken in fruit flowers, transfer a certain amount of pollen with which their bodies become covered as they move slowly from one flower to another when seeking nectar and aphides. *Adalia bipunctata*, with its wide range of colour variation, and *Coccinella septempunctata*, which is constant in its markings, were taken in ten and eight different fruit flowers respectively. The former, besides satisfying itself with a liquid diet, was observed to feed on the following species of aphides as they crawled over the petals: *Anuraphis roseus* (apple), *A. helichrysi* (plum), *Myzus cerasi* (cherry) and *Amphorophora rubi* (raspberry).

**Fam. Nitidulidae.** A large number of species belonging to this family are anthophilous, and the most numerous of all beetles found in fruit blossom was *Meligethes aeneus*. As many as nine beetles were seen in one pear blossom where they were observed to lick the nectar and devour the floral organs.

**Fam. Scarabaeidae.** The three members of this family, with the possible exception of one (*Aphodius inquinatus*) are useless as pollinating agents. The cockchafer, *Melolontha vulgaris*, and the garden chafer, *Phyllopertha horticola*, are phytophagous, and a great amount of damage to the flowers of apple, quince and other fruit trees is recorded. Several individuals of a dung beetle, *A. inquinatus*, covered with pollen have been observed crawling over the flowers of apple and pear and licking the nectar in them.

**Fam. Elateridae.** The presence of "click-beetles" on flowers is aggravated by the damage they do by eating the petals and anthers. One species, *Limonijs cylindricus*, whose larvae is an injurious "wireworm," has frequently been seen to damage the flowers of apple and red currant. During the spring of 1924 many individuals were taken from apple blossom and not, infrequently, two beetles in one flower, the petals of which were speedily reduced to ribbons.

**Fam. Cerambycidae.** The wasp-beetle, *Clytus arietis*, and *Strangalia armata* were the sole representatives of this extensive family of longicorns taken on fruit flowers. Their visits were restricted to blackberry and loganberry, but an occasional wasp-beetle was seen licking the nectar of red currant flowers. They feed on nectar and pollen, but the amount transferred by either is negligible. *S. armata* will often devour the petals and anthers.

**Fam. Chrysomelidae.** *Cassida viridis*, one of the "tortoise" beetles, was an occasional visitor to apple and pear flowers, but it remains too long in one flower



to warrant its inclusion amongst the useful agents. *Lema melanopa*, a beetle injurious to growing cereals, proved to be of little use as an attendant on fruit blossom.

**Fam. Curculionidae.** The four species of weevils recorded from fruit blossoms are all injurious either in the larval and adult stages or in both stages to fruit, and their absence rather than their presence is preferred. The apple blossom weevil, *Anthonomus pomorum*, is a common inhabitant of most orchards and its control is of serious import to fruitgrowers. It has been observed that both sexes, prior to mating, feed by perforating the petals of fully expanded flowers and young foliage. The female oviposits in the unopened blossoms of apple and, to a less degree, pear.

*Rhynchites aequatus* is a periodic pest of some varieties of apple in orchards in Surrey and Kent. The larval damage is confined to the fruitlets, but the adults perforate the petals of apple and quince and set up a speedy decay of the floral organs.

Two species of *Phyllobius* have been taken on apple blossom. *P. oblongus* was also taken on black currant, whilst *P. pyri*, the more injurious species, committed great havoc by devouring the floral organs and foliage on apple, pear and quince.

#### Order LEPIDOPTERA.

For the sake of convenience the old and more familiar divisions into RHOPALOCERA and HETEROCERA are used. Most members of this order possess trophi adapted to a liquid diet. Rosaceous flowers with their shallow corolla do not prove attractive to many butterflies and moths, hence the few records we possess of species visiting fruit blossoms. The fruit plantations at Wisley and elsewhere have been examined at various times during the flowering period of the several fruits between 8 p.m. and 1 a.m., but comparatively few moths have been seen on the flowers. So far as our observations go, there is no evidence to suggest that night-flying moths play any serious part in the pollination of fruit blossoms, although it has been suggested that moths may contribute their quota(8).

#### Sub-order RHOPALOCERA.

**Fam. Nymphalidae.** *Vanessa io* is the only member of this family that has on occasions been seen imbibing the nectar of apple and plum flowers. Hibernated specimens emerge during warm days in early spring at a time when there are few "butterfly" flowers open. The insect stands on the fully expanded corolla and thrusts its proboscis down into the nectaries, but its body rarely comes into contact with the stamens and pistil, so that the amount of pollen transferred by it is infinitesimal.

**Fam. Pieridae.** The small cabbage white, *Pieris rapae*, and the green-veined white, *P. napi*, have been observed to visit the flowers of apple and strawberry and cherry and blackberry respectively. From the amount of pollen found on their bodies, their presence on fruit flowers is useless to the fruitgrower.

#### Sub-order HETEROCERA.

**Fam. Hydrimenidae.** One species of *Eupethestia* (the specimens were too much rubbed to determine with certainty) was taken on black currant and gooseberry flowers. The moths were seen flitting among the bushes on dull days. It is not uncommon to find the larvae of the green-pug moth, *Chloroclystis rectangulata*, in apple and quince flowers, which they destroy. They eat the petals and anthers with avidity.

Fam. **Caradrinidae**. *Anarta myrtili* was observed to suck the nectar from red currant flowers on bright sunny days in 1924.

#### Order **HEMIPTERA**.

##### Sub-order **HETEROPTERA**.

Fam. **Pentatomidae**. One of the carnivorous shield-bugs, *Pentatoma rufipes*, was occasionally found crawling over the flowers of loganberry in search of prey, but its slow rate of progress eliminates it as a pollinating agent of any importance.

Fam. **Anthocoridae**. The aphidivorous species, *Anthocorus nemorum*, was taken on apple, raspberry and red currant flowers in and around which it made its way to search for aphides on which it fed.

##### Sub-order **Homoptera**.

Fam. **Aphididae**. Certain species of aphides with their bodies covered in pollen have at times been taken from fruit blossom. The commonest species being *Anuraphis roseus*, which often clusters on the inside and outside of apple petals. The other species taken as they were crawling over the floral organs were the leaf-curling plum aphid, *Anuraphis helichrysi*, *Amphorophora rubi* on raspberry and *Aphis grossularia* in the flowers of red currant and gooseberry.

#### Order **THYSANOPTERA**.

Members of this order are grouped with the family *Aphididae* in not possessing sufficiently industrious habits to warrant their inclusion as desirable pollinators. Thrips were found in abundance in apple and pear blossom and, although they distribute a certain amount of pollen as they travel from one flower to another in search of nectar, they derive a great amount of nourishment by puncturing the tissues of the corolla with their piercing mouthparts.

#### Order **DERMAPTERA**.

Fam. **Forficulidae**. The common earwig, *Forficula auricularia*, is a nocturnal feeder and has been found licking nectar in plum and blackberry flowers. A great amount of damage to plum flowers in 1922 was occasioned by these insects through the destruction of the petals and stamens. Besides their herbivorous habits, they confine their attention to a single cluster of blossom in which they remain until the floral organs are reduced to fragments. Apple blossom is likewise damaged by these insects.

#### Order **ARANEIDA**.

Spiders visit flowers for no other purpose than the capture of insects as prey. They crawl over the flowers and some species spin webs round the clusters and, in so doing, capture many pollinating insects, chiefly small flies (species of *Chironomus* and *Sciara* and Anthomyiids). Their search continues by night as well as by day, and a small amount of pollen is thus carried by them.

Fam. **Epeiridae**. One of the orb-weavers was well represented on apple, pear and plum blossom, over clusters of which is spun the snare. Individuals were also observed to crawl over the flowers of currants and gooseberry, but no web was spun among the blossoms.

Fam. *Thomasidae*. A species of wolf-spider was seen occasionally to lie in wait inside apple and pear flowers and capture the smaller species of anthophilous insects.

Fam. *Salticidae*. The common British jumping-spider, *Saliculus scenicus*, did on occasion wander over apple and pear blossoms, behind the corolla of which it lurked in search of insect prey.

10. CENSUS OF CHIEF POLLINATING AGENTS OF HARDY FRUIT AT WISLEY,  
SURREY, TOGETHER WITH METEOROLOGICAL DATA (RECORDED—  
9 A.M.) OF THE PERIOD DURING WHICH OBSERVATIONS WERE MADE.

APPLE.

			1920 April 12– May 1 12 h. 40 m.	1921 April 8– May 13 12 h. 25 m.	1922 May 9– 26 7 h. 15 m.	1923 April 23– May 13 5 h. 10 m.	1924 May 14– 17 1 h. 15 m.
<b>HYMENOPTERA</b>							
Hive bees	...	...	0	85	74	48	15
Humble bees	...	...	103	134	55	55	10
Wild bees	...	...	10	30	41	16	9
Others	...	...	6	10	24	3	1
<b>DIPTERA</b>							
Hover flies	...	...	14	135	46	21	9
Bluebottles	...	...	8	11	28	14	3
Others*	...	...	45	55	48	31	20
<b>COLEOPTERA</b>							
Beetles and weevils	...	...	9	30	27	1	12
<b>LEPIDOPTERA</b>							
Butterflies and moths	...	...	3	4	5	5	1
<b>PLECOPTERA</b>							
Stone flies	...	...	0	0	1	0	0
<b>MECOPTERA</b>							
Scorpion flies	...	...	0	0	2	1	0
<b>HEMIPTERA</b>							
Bugs and aphides	...	...	2	0	0	0	3
<b>THYSANOPTERA</b>							
Thrips	...	...	2	5	4	7	0
<b>ARACHNIDA</b>							
Spiders	...	...	3	3	1	0	1
<hr/>							
Mean max. temp. of air (° F.)	...	...	56.02	58.95	69.55	60.68	68.00
Mean min. temp. of air	...	...	42.74	39.30	46.64	42.90	43.87
Mean min. temp. on grass	...	...	34.50	29.52	37.76	34.42	33.50
Mean temp. of soil 1 ft. down	...	...	49.59	50.37	56.53	55.49	57.70
No. of ground frosts	...	...	7	20	4	8	1
No. of air frosts	...	...	0	6	1	2	0
Amount of sunshine (hr.)	...	...	72.3	221.1	162.9	129.0	39.2
Amount of rainfall (in.)	...	...	1.97	1.45	0.56	1.05	0.02
Prevailing wind	...	...	S.W.	N.W. April S.W. May	S.W.	S.W.	S.W.

\* Exclusive of *Sciara* species.



## CHERRY.

				1922 May 8-9 1 h. 0 m.	1923 April 18- May 14 1 h. 35 m.	1924 May 14 0 h. 30 m.
<b>HYMENOPTERA</b>						
Hive bees...	...	...	...	4	22	5
Humble bees	...	...	...	9	19	3
Wild bees...	...	...	...	8	1	0
Others	...	...	...	3	0	1
<b>DIPTERA</b>						
Hover flies	...	...	...	3	3	1
Bluebottles	...	...	...	5	2	0
Others*	...	...	...	0	2	1
<b>COLEOPTERA</b>						
Beetles and weevils	...	...	...	2	0	1
<b>LEPIDOPTERA</b>						
Butterflies	...	...	...	1	1	0
Mean max. temp. of air (° F.)	...	...	...	77.90	59.11	73.20
Mean min. temp. of air	...	...	...	45.65	42.21	48.90
Mean min. temp. on grass	...	...	...	35.60	33.85	38.00
Mean temp. of soil 1 ft. down	...	...	...	54.40	52.22	57.30
No. of ground frosts	...	...	...	0	9	0
No. of air frosts	...	...	...	0	2	0
Amount of sunshine (hr.)	...	...	...	22.0	156.5	7.9
Amount of rainfall (in.)	...	...	...	0	1.1	0.01
Prevailing wind	...	...	...	N.W.	S.W.	S.W.

## PLUM.

				1920 March 25- April 8 3 h. 0 m.	1921 March 10- April 13 3 h. 23 m.	1922 April 21- May 9 9 h. 40 m.	1923 March 26- April 24 8 h. 15 m.
<b>HYMENOPTERA</b>							
Hive bees	...	...	...	0	15	18	6
Humble bees	...	...	...	34	56	135	83
Wild bees	...	...	...	0	21	46	23
Others	...	...	...	0	1	5	2
<b>DIPTERA</b>							
Hover flies	...	...	...	1	44	31	13
Bluebottles	...	...	...	2	15	33	18
Others*	...	...	...	5	18	25	22
<b>COLEOPTERA</b>							
Beetles and weevils	...	...	...	5	2	4	1
<b>LEPIDOPTERA</b>							
Butterflies and moths	...	...	...	1	0	1	0
<b>HEMIPTERA</b>							
Aphides	...	...	...	0	2	0	0
<b>ORTHOPTERA</b>							
Earwigs	...	...	...	0	0	0	2
<b>ARACHNIDA</b>							
Spiders	...	...	...	1	1	0	0
Mean max. temp. of air (° F.)	...	...	...	54.82	57.35	57.93	55.27
Mean min. temp. of air	...	...	...	43.31	41.63	37.96	40.34
Mean min. temp. on grass	...	...	...	36.29	27.76	29.47	32.24
Mean temp. of soil 1 ft. down	...	...	...	47.87	46.33	48.00	48.50
No. of ground frosts	...	...	...	4	23	11	10
No. of air frosts	...	...	...	0	4	3	3
Amount of sunshine (hr.)	...	...	...	31.0	209.6	131.0	125.0
Amount of rainfall (in.)	...	...	...	1.48	1.28	1.19	1.75
Prevailing wind	...	...	...	S.	S.W. March N.E. April	N.W.	N.E.

\* Exclusive of *Sciara* species.

## LOGANBERRY.

## RASPBERRY.

	1923 June 13- July 1 2 h. 35 m.	1924 June 17- 19 0 h. 35 m.	1923 June 6- July 1 0 h. 45 m.	1924 June 11- 19 1 h. 30 m.
<b>HYMENOPTERA</b>				
Hive bees ...	33	4	4	9
Humble bees ...	24	5	13	19
Wild bees ...	10	1	6	7
<b>DIPTERA</b>				
Hover flies ...	27	13	3	5
Bluebottles ...	0	1	—	—
Others ...	0	2	6	4
<b>COLEOPTERA</b>				
Beetles ...	2	2	2	1
<b>LEPIDOPTERA</b>				
Moths ...	—	—	1	1
<b>HEMIPTERA</b>				
Bugs ...	0	1	1	—
Aphides ...	—	—	—	3
Mean max. temp. of air (° F.)	65.24	72.23	64.90	67.34
Mean min. temp. of air ...	48.52	52.17	48.37	49.77
Mean min. temp. on grass ...	40.46	43.43	40.82	43.37
Mean temp. of soil 1 ft. down	52.01	53.93	57.50	61.77
No. of ground frosts ...	1	0	2	0
No. of air frosts ...	0	0	0	0
Amount of sunshine (hr.) ...	80.0	31.0	112.1	72.2
Amount of rainfall (in.) ...	0.26	0.38	0.30	0.96
Prevailing wind ...	W.	S.W.	W. partly S.W. partly	S.W.

## BLACK CURRANT.

	1920 April 8 0 h. 10 m.	1923 April 5- May 1 3 h. 25 m.	1924 April 23- May 18 2 h. 45 m.
<b>HYMENOPTERA</b>			
Hive bees ...	0	0	9
Humble bees ...	11	46	31
Wild bees ...	0	3	10
Others ...	1	3	3
<b>DIPTERA</b>			
Hover flies ...	1	5	5
Bluebottles ...	1	4	3
Others* ...	1	6	6
<b>COLEOPTERA</b>			
Beetles and weevils ...	0	3	1
<b>LEPIDOPTERA</b>			
Moths ...	0	0	1
<b>ARACHNIDA</b>			
Spiders ...	0	1	0
Mean max. temp. of air (° F.) ...	56.00	54.24	59.86
Mean min. temp. of air ...	47.50	39.83	44.16
Mean min. temp. on grass ...	46.30	32.08	37.43
Mean temp. of soil 1 ft. down ...	48.70	48.73	52.93
No. of ground frosts ...	0	10	5
No. of air frosts ...	0	3	0
Amount of sunshine (hr.) ...	0	110.8	125.6
Amount of rainfall (in.) ...	0.10	2.17	3.52
Prevailing wind ...	S.W.	N.E.	S.W.

\* Exclusive of *Sciara* species.

## RED AND WHITE CURRANTS.

	1920 April 8 0 h. 10 m.	1922 April 28- May 9 0 h. 50 m.	1923 April 11- 26 1 h. 55 m.	1924 April 28- May 18 2 h. 25 m.
<b>HYMENOPTERA</b>				
Hive bees ... ..	0	4	0	2
Humble bees ... ..	3	5	10	2
Wild bees ... ..	0	3	4	30
Others ... ..	1	1	2	2
<b>DIPTERA</b>				
Hover flies ... ..	1	5	5	9
Bluebottles ... ..	0	2	10	8
Others* ... ..	1	1	15	18
<b>COLEOPTERA</b>				
Beetles ... ..	0	3	0	8
<b>LEPIDOPTERA</b>				
Moths ... ..	0	0	0	3
<b>HEMIPTERA</b>				
Bugs and aphides ... ..	0	0	2	2
<b>ARACHNIDA</b>				
Spiders ... ..	0	0	2	1
Mean max. temp. of air (° F.) ...	56.00	61.20	54.00	60.54
Mean min. temp. of air ...	47.50	38.88	39.04	43.76
Mean min. temp. on grass ...	46.30	29.59	32.15	36.54
Mean temp. of soil 1 ft. down ...	48.70	49.05	48.56	53.42
No. of ground frosts ... ..	0	7	5	5
No. of air frosts ... ..	0	3	1	0
Amount of sunshine (hr.) ...	0	95.5	70.4	115.7
Amount of rainfall (in.) ...	0.10	0.49	1.59	2.35
Prevailing wind ... ..	S.W.	S.W.	N.E.	S.W.

## GOOSEBERRY.

	1920 April 8 0 h. 10 m.	1922 April 28- May 9 0 h. 55 m.	1923 April 9- 26 3 h. 20 m.	1924 April 24- May 11 1 h. 0 m.
<b>HYMENOPTERA</b>				
Hive bees ... ..	0	3	0	2
Humble bees ... ..	1	4	38	9
Wild bees ... ..	0	0	10	1
Others ... ..	1	4	4	2
<b>DIPTERA</b>				
Hover flies ... ..	0	3	3	0
Bluebottles ... ..	2	3	3	1
Others* ... ..	6	9	8	2
<b>COLEOPTERA</b>				
Beetles and weevils ... ..	0	3	1	0
<b>LEPIDOPTERA</b>				
Moths ... ..	0	0	0	1
<b>HEMIPTERA</b>				
Aphides ... ..	0	0	1	1
<b>ARACHNIDA</b>				
Spiders ... ..	0	0	2	0
Mean max. temp. of air (° F.) ...	56.00	61.20	53.43	57.30
Mean min. temp. of air ...	47.50	38.88	38.44	43.00
Mean min. temp. on grass ...	46.30	29.59	30.34	36.55
Mean temp. of soil 1 ft. down ...	48.70	49.05	48.12	51.40
No. of ground frosts ... ..	0	7	7	4
No. of air frosts ... ..	0	3	3	0
Amount of sunshine (hr.) ...	0	95.5	74.9	77.5
Amount of rainfall (in.) ...	0.10	0.49	1.91	3.08
Prevailing wind ... ..	S.W.	S.W.	N.E.	S.W.

\* Exclusive of *Sciara* species.

## 11. SUMMARY.

It was considered necessary to carry out observations on the insects concerned in the pollination of hardy fruit flowers over long periods in order to arrive at definite conclusions as to what insects may be considered essential for the carrying out of the work.

The result of five years' work has shown the usefulness of the hive bee as a pollinating agent, yet the important work of pollination, under certain conditions, may be carried out entirely by wild insects, principally humble and other wild bees (*Bombus* and *Andrena*) and flies (*Eristalis*, *Syrphus*, *Sciara*, Anthomyiids and *Calliphora*).

The district and the position of orchards govern to a large extent the numbers of wild insects, and the plantations at Wisley are favoured in this respect by their close proximity to open country and pasture land.

The factors, other than the presence of pollinating agents, which influence fruitfulness in orchards are considered.

The transference of pathogenic organisms by anthophilous insects is discussed.

The species of insects taken on the various fruit flowers are listed together with data as to their individual habits and abundance.

A census of the chief pollinating agents of hardy fruit flowers at Wisley is appended and the meteorological data covering the period over which observations were made.

## REFERENCES.

- (1) BACKHOUSE, W. O. (1912). *Gardeners' Chronicle*, 23rd November, p. 381.
- (2) BARKER, B. T. P. and GROVE, O. (1914). *Ann. App. Biol.* i, 85-97.
- (3) BOND, C. J. (1921). *Nature*, 7th July, p. 584.
- (4) CHAPMAN, T. A. (1878). *Entomologist's Monthly Magazine*, xiv, 196-200.
- (5) CHITTENDEN, F. J. (1914). *Ann. App. Biol.* i, 41-42.
- (6) DOIDGE, E. M. (1917). *Ibid.* iv, 50-74.
- (7) DORSEY, M. J. (1919). *Journ. Agric. Res.* xvii, 103-26.
- (8) DURHAM, H. E. (1921). *Gardeners' Chronicle*, 6th August, p. 78.
- (9) EDWARDS, F. W. (1925). *Ann. App. Biol.* xii, 263.
- (10) FRISCH, K. v. (1923). *Zool. Jahrb. Jena, Abt. f. allg. Zool.* xl, 1-186.
- (11) — (1924). *Sinnesphysiologie und Sprache der Bienen* (Berlin).
- (12) — (1927). *Naturwissenschaften*, xv, 321-27.
- (13) HATTON, R. G. (1921). *Journ. Pomology*, ii, 160-98.
- (14) HOOPER, C. H. (1911). *R.H.S. Journ.* xxxvi, 548-64.
- (15) — (1913). *Ibid.* xxxviii, 244-46.
- (16) — (1919). *Journ. Pomology*, i, 116-24.
- (17) HUTSON, RAY (1926). *New Jersey Agric. Exp. Stat. Bull.* 434, pp. 2-32.
- (18) KNUTH, P. (1906). *Handbook of Flower Pollination*. 3 vols.



- (19) LEWIS, C. I. and VINCENT, C. C. (1909). *Oregon Agric. Exp. Stat. Bull.* 104, pp. 3-40.
- (20) LOVELL, J. H. (1920). *The Flower and the Bee*, p. 213.
- (21) LUTZ, F. E. (1924). *Ann. Acad. Sciences*, xxix, 233-283.
- (22) McINDOO, N. E. and DEMUTH, G. S. (1926). *U.S. Dept. Agric. Bull.* 1364, pp. 1-32.
- (23) MÜLLER, H. (1879). *Weitere Beobachtungen über Befruchtung der Blumen durch Insekten*, II, 244 and 288. (Berlin.)
- (24) — (1883). *The Fertilisation of Flowers*.
- (25) REID, W. F. (1910). *R.H.S. Journ.* xxxv, 195-203.
- (26) WALTON, C. L. (1922). *Entomologist's Monthly Magazine*, 2nd Ser. viii, 271-75.
- (27) — (1927). *Ann. App. Biol.* xiv, 465-69.
- (28) WILSON, G. FOX (1926). *R.H.S. Journ.* li, 225-51.

(Received April 12th, 1929.)

## THE LARVA AND PUPA OF *SCATOPSE FUSCIPES* MG. AND A COMPARISON OF THE KNOWN SPECIES OF SCATOPSID LARVAE

By EDITH LYALL, B.Sc.

(*Demonstrator in Entomology.*

*Department of Entomology, Imperial College of Science and Technology.*)

(With 14 Text-figures.)

DURING the investigation of insects injurious to stored products in progress at the Imperial College, certain dipterous larvae of the family Scatopsidae were obtained living in green ginger which had been damaged by water. These larvae were accompanied by adult flies which were identified by Mr O. W. Richards as *Scatopse fuscipes* Mg. It was felt that descriptions of the larvae and pupae might be useful in the stored products research, and, as there appeared to be no complete description of Scatopsid larvae, it was suggested that a description of the general morphology of the larva and pupa of *S. fuscipes* should be made.

The material studied was obtained from green ginger at the Metropolitan Wharf, Wapping, in the autumn of 1927. The ginger was unfit for consumption and probably harboured moulds or similar fungi which, together with the *Scatopse* larvae, made it soft, wet and spongy with a marked odour resembling citrus. Larvae and pupae of all stages were found and adult flies were reared.

### PREVIOUS WORK ON SCATOPSID LARVAE.

The larvae of only two species of Scatopsid have been described previously. *Scatopse notata* was first described, according to the literature cited by Morris (1918), as early as 1776 by Degeer under the name of *Tipula latrinarum*. It was again described by Bouche, 1834, as *Scatopse noir* Geoffr. and by Perris (1847) as *Scatopse punctata* Meig. None of these descriptions was accurate. Dufour (1846) described a larva of *Scatopse nigra* which is thought to be that of *S. fuscipes* Mg. He thought that the larva was amphineustic, not recognising the intermediate abdominal spiracles as such. He mistook the mandibles for maxillae and decided that it had no mandibles. Perris did not make this mistake,

but did not see the maxillae. Both authors describe the labrum. Dufour begins by saying that the body has eleven segments and states later that the eleventh segment consists of two fused, so that there are really twelve. This is the true state of affairs, the dorsal suture being difficult to see.

De Meijere (1917) describes the larva of *Scatopse notata* L. in great detail. He mentions the peculiar labium and hypopharynx but evidently did not see the premandibles and triangular pieces of the labrum. He attaches much importance to the setal pattern, both dorsal and ventral.

H. M. Morris (1918) describes the larva of *S. notata* and compares it with that of *Bibio johannis*. He does not describe the mouth parts other than by a series of drawings which do not agree in detail with my observations.

He lays greatest stress upon the arrangement of the setae. The setal pattern should be of great importance in the classification of these larvae. He describes the pupa.

G. W. Müller (1919) obtained a Scatopsid, *Reichertella femoralis* Mg., from a pupa of *Phora*. This is the only mention of a parasitic Scatopsid.

F. W. Edwards (1925), in his key to the species of *Scatopse*, notes the larval habits of those whose larvae have been recognised. The larvae appear to be saprophagous; they are found on a variety of organic substances all in a state of putrefaction; *S. notata* L. is found commonly in dung, it has also been found in rotten onions (British Museum specimens), and in an old, decaying wasp's nest among dead leaves (Morris, 1918). *Scatopse fuscipes* larvae are recorded as feeding in dung and rotten onions.

Until 1926 nothing seems to have been known of the larvae of species other than *S. fuscipes* and *S. notata*, when M. Tonnoir (1926) described a third species *S. subnitens* Verr. (*S. nigra* Mg.). This species was found under the bark of felled poplars at Hoogstraeten, Antwerp. He gives a general description, emphasising the dorsal setal pattern and the fact that the last larval skin is retained as a covering for the pupa. He does not describe the mouth parts.

It will be seen from the above that the work done on these larvae is meagre and very little is known of the life history of most of them.

#### *SCATOPSE FUSCIPES* Mg.

*Larva.* The general aspect of the larva is very similar to that of *Scatopse notata* as described by Morris and of *S. subnitens* as described by Tonnoir. It is considerably smaller than *S. notata*, measuring only  $2\frac{1}{2}$  mm. as compared with 7 mm., the length of *S. notata*.

The head capsule is strongly chitinised. It is bluntly pointed in front, the sides of the head being subparallel. The dorsal surface of the head is almost smooth in the middle but is sculptured laterally. The labrum is marked by several transverse ridges and indeterminate scrolling and bears, about the middle of its length, two small tufts of hairs. The head bears a pair of tufts on each side, the outer tuft consisting of one or two hairs only. The sutures between the frons, clypeus and labrum are not discernible.

The sides of the head capsule are bent under the head, so that they almost meet, leaving only a narrow space between the inner margins; these flaps are the only ventral chitinisation. They taper towards one another and immediately in front of them lies the labium.

There are no eyes.

The antenna consists of three segments. The basal narrow, ring-like and appearing as a swelling on the head; the second also narrow and ring-like but of much smaller circumference; and the third long and finger-like. The second segment bears on its outer or upper surface a spike-like projection similar in form to the third segment, but only about one-third of its width, and about three-quarters of its length. The projection is slightly clavate. The second joint also bears on its upper surface three shorter projections standing side by side. See Fig. 2.

The body of the larva is white and cylindrical, widest in the middle and tapering slightly towards the ends. There is no external difference between the segments of the thorax and the abdomen. There are twelve segments behind the head, the dorsal part of the eleventh covering the twelfth, so that only a short semicircular piece of the twelfth is seen in dorsal view (Fig. 11). In ventral view (Fig. 12) the twelfth is wholly visible and equal in length to the eleventh. The surface of the body is covered with setae which are of various types. There are short stout setae scattered over the surface and longer fine ones which are arranged in an irregular pattern which does not appear to be constant on each segment. In the posterior region of the larva the setae become longer and coarser, while those surrounding the anus are almost bristle-like. The pattern on the first segment, however, is more definite than that on the others and appears to be constant. There is a well-defined transverse row of prominent setae on the proximal border of the seventh to tenth segments on the dorsal surface. The row of setae on the eleventh lies in the middle of the segment and there are no other large setae on this segment (Fig. 1). The sides of the larva are fringed by hairs which increase in length and thickness on the posterior segments.

There are nine pairs of spiracles, borne on the first, and on the fourth to eleventh segments. The spiracles project laterally as small chitinised papillae, wider at the apex than at the base. Those on the eleventh segment are placed on the posterior border of the segment and their papillae are considerably longer than those of the other spiracles. At the apex of the last pair of spiracles is a circular collar bearing a fringe of setae. The trachea can be distinctly seen passing down the papilla and forking as soon as it enters the eleventh segment, one branch apparently entering the twelfth segment (see Figs. 11, 12).

The twelfth segment bears two spiracle-like projections on its caudal border. These projections are chitinised on the dorsal surface only and are fringed with long coarse setae. The anus lies between these projections on the ventral surface. It is longitudinal and is surrounded by a series of membranous lobes which, while protecting the opening, allow of its enlargement when necessary. The anus is further protected by a single row of stout setae which lie outside the anal lobes and project over them.

*Mouth-parts.* The mouth-parts are well developed.

The labrum (Figs. 3, 4, 5) is narrow in front, the sides sloping away from each other behind. It is sculptured on the dorsal surface, but on the ventral surface the labrum is composed of a trapezoidal, lightly chitinised portion, the narrower margin being posterior. This posterior margin widens out into a semicircular piece—the epipharynx, which is lightly chitinised and is bounded by two half hoops of chitin which almost meet at the apex, this hoop being called by Goetghebuer “la pièce en U” or the U-shaped piece.

The *labrum* is supported by two lateral chitinised plates called by the same author “Les pièces triangulaires.” The labrum and epipharynx bear numerous irregularly quadrangular, convex, chitinous swellings, and these in their turn bear numerous long setae projecting posteriorly. The setae project as a fringe around the edge of the epipharynx (Fig. 4). Articulating with the lateral triangular pieces and lying in the membrane are two heavily-chitinised structures, oval and bearing on the margin nearest the epipharynx four large blunt teeth. These are termed by Goetghebuer “premandibles.” He describes them in several families of Nematocerous Diptera and in *Scatopse flavicollis* Meigen, as having only three teeth (Figs. 4 and 5).

In *Scatopse fuscipes* they clearly have four teeth and in *S. notata* (Fig. 6) they appear to have five, the fourth tooth being divided into two fine teeth. These premandibles are extraordinarily mobile and, according to Goetghebuer, can move in the antero-posterior plane as well

as in the transverse plane. Edwards (1925) considers that these are primitive structures retained independently in a number of families and lost in the others. They occur in Scatopsidae but not in Bibionidae. This is one of the reasons for separating the Scatopsidae as a family from the Bibionidae.

*The mandible.* The apical part of the mandible (Figs. 3 and 4) is shaped like a shallow scoop, the base of which at one side is much widened. The scoop is bordered by seven large blunt teeth. Viewed ventrally the wide base of the mandible bears on the apex of its inner margin two teeth, a large and a small. The base of the mandible is hollow, while the outer side below the teeth bears a row of lamellate setae which decrease in size towards the ventral surface. Below these is a single long projection veined like a leaf. At the base of the mandible is a tuft of small setae (Figs. 7 and 8). These mandibles work in the transverse plane and when horizontal conceal the epipharynx.

*The maxillae* (Fig. 9) overlie the bases of the mandibles in the ventral view. They are more or less membranous and are supported by several chitinised plates. They consist of an outer lobe, which probably represents a one-jointed palp and a wide inner lobe bearing several chitinous teeth on its anterior margin and numerous long setae. The palp carries one circular and one semicircular piece of chitin enclosing numerous small papillae.

*The labium* (Fig. 10) consists of a semicircular plate, the posterior margin being straight and fringed irregularly with long setae. This plate shows on its ventral surface two crescent-shaped chitinous pieces bearing numerous papillae within their curves. Anterior to the labium is another plate, also semicircular but bearing on each side laterally a chitinous arm, which projects obliquely under the maxillae. This plate shows numerous irregular strongly chitinised areas. Miall and Hammond, when describing the labium of the Chironomous larva, suggest that the anterior plate is the mentum which has partially slipped down behind the posterior plate—the submentum. This seems doubtful. Morris in his description of the larvae of species of *Bibio* shows a labium consisting of two plates, but does not name them separately. Imms (1925) calls the anterior plate the hypopharynx, and this would seem to be the most probable homology.

The relative positions of the mouth-parts in ventral view is made clear in Fig. 3.

*The pupa.* (Figs. 13 and 14.)

The larval skin remains as a covering for the pupa. The pupa is widest in the middle and tapered slightly at its extremities, the posterior end being narrower. It is brown, 2 to  $2\frac{1}{2}$  mm. long and  $\frac{1}{2}$  to  $\frac{3}{4}$  mm. wide at its broadest part.

There are six pairs of abdominal spiracles placed laterally on the second to seventh segments. Their papillae are considerably longer than those of the larva and project through the larval skin. On the dorsal surface of the prothorax are a pair of long forked spiracles. The centre of the spiracular structure is strongly chitinated, and in the less chitinated part are numerous laterally-placed chitinous projections which decrease in size towards the apices of the structure. The base of the structure is slightly swollen.

Between the two prothoracic spiracles is a diamond-shaped feebly chitinated area. It is here that the pupal skin begins to rupture before the emergence of the imago, the thorax splitting down the mid-line from this area.

On the ventral surface the antennal sheaths can be seen extending on each side to the base of the wing. The sheaths of the first two pairs of legs meet in the mid-line, the third pair do not meet and all but the apices are hidden by the wing sheaths. The third pair of leg sheaths extend to the posterior margin of the first abdominal segment. The sheaths of the labrum and of the maxillary palps can also be seen, closely attached to the body.

There are eight abdominal segments. The individual segments of the thorax are not clearly indicated.

COMPARISON OF THE LARVAE OF THE THREE SPECIES, *S. fuscipes*, Mg.,  
*S. notata*, L. AND *S. subnitens*, VERR.

In the three species the larvae differ greatly in size, *S. fuscipes* measuring  $2\frac{1}{2}$  mm., *S. subnitens*,  $3\frac{1}{2}$  mm. and *S. notata* 7 mm.

The chief point of difference in the heads of the three species lies in the antennae. The length of the spike borne on the second segment varies in the three species. In *S. fuscipes* the spike is  $\frac{3}{4}$  the length of the third segment, in *S. notata* it is about  $\frac{1}{3}$  the length and in *S. subnitens* about  $\frac{1}{2}$  the length of the third joint. This difference is very marked.

Morris does not describe the mouth-parts of *S. notata* other than by a series of drawings—these drawings differ in several details from the description of *S. notata* which follows:

The mouth-parts are very similar to those of *S. fuscipes* and lateral triangular pieces and premandibles are present. The premandibles of

*S. notata* have five teeth, the posterior two being very fine. Those of *S. fuscipes* appear to have only four. The labrum is very similar in both species, though the chitinous arms of the hypopharynx in *S. notata* are wider than those of *S. fuscipes*. The mandibles of the two species are similar, as are also the maxillae, the chief difference being in the size. Morris's drawings do not accurately indicate the position of the chitinous plates.

The mouth-parts of *S. subnitens*, as described by Tonnoir, show only that the ventral surface of the labrum bears fine spinules pointing towards the posterior and that the labrum is truncated. It seems possible that they may be similar in essentials to those of *S. fuscipes*.

The only striking differences in the bodies of the larvae lie in the arrangement of the large setae on the dorsal surface of the segments. Those on *S. fuscipes* do not form a definite constant pattern. Those on *S. notata* do, as also do those on *S. subnitens*. The setae on the thorax of the latter species are differently arranged from those on the abdominal segments. There is a median row on all the segments which is doubled on itself in the thoracic segments. The two lateral rows diverge from behind forwards on the thoracic segments and the three lateral rows diverge from before backwards on the abdominal segments.

In *S. notata* the setae are arranged in five longitudinal rows on each segment, except the eleventh, there being little difference in arrangement in any of the segments. On the eleventh there is a single median transverse row of setae which at the sides is continued back to the posterior margin. The anus is protected in *S. notata* by two rows of setae, the long setae around the anal opening and a row of shorter ones on each side. That of *S. fuscipes* is protected by a single row only.

The pupa of *S. fuscipes* differs from that of *S. notata* mainly in size, that of *S. notata* being 4 mm. and that of *S. fuscipes* 2-2½ mm. long. The prothoracic spiracular structures of *S. notata* are shorter and are not forked but bear several short branches.

Tonnoir does not give the length of the pupa of *S. subnitens*, but from his drawing the prothoracic spiracles are apparently similar to those of *S. fuscipes*.

The important differences between the three species seem to lie in the length of the antennal spike and in the setal pattern on the dorsal surface of the larva and to some extent in the shape of the prothoracic stigmata of the pupa.



## SUMMARY.

The larvae of *Scatopse fuscipes* were found in decaying green ginger at a London dock in 1927. The necessity for descriptions of larvae occurring in stored products is pointed out.

The previous literature is discussed. General descriptions of the larvae and pupae are given, especial attention being paid to the mouth-parts of the larvae, which have in common with larvae of several Dipterous families, "premandibles."

A short comparison of the larvae of *S. fuscipes*, *S. notata* and *S. subnitens* is made and the most important differences between them are emphasised. No other species have yet been described.

I have to express my indebtedness to Mr O. W. Richards for material and references and to Dr J. W. Munro for his assistance and advice.

## REFERENCES.

- (1) DUFOUR, L. (1846). Histoire des Métamorphoses du *Scatopse noir* Geoffr. *Ann. Sci. Nat.* Sér. iii, t. 6.
- (2) DE MEIJERE, J. C. H. (1917). Beitrage zur Kenntnis der Dipteren-Larven und Puppen. *Zool. Jahr. Syst.* XL, 180.
- (3) EDWARDS, F. (1925). Synopsis of British Bibionidae and Scatopsidae. *Ann. App. Biol.* XII, 263-75.
- (4) — (1925). The Phylogeny of Nematocerous Diptera: a critical review of some recent suggestions. *Verhandlungen des III Internationalen Entomologen-Kongresses Zurich*, 1925. Band I and II.
- (5) GOETGHEBUER, Dr M. (1926). *Encyclopédie Entomologique*, Série B. II. *Diptera*, tome I, 1924, pp. 143-57.
- (6) IMMS, A. D. (1925). *Text-book of Entomology*, 1926, p. 609.
- (7) MIALL, L. C. and HAMMOND, A. R. (1900). *The Harlequin Fly*, p. 27.
- (8) MORRIS, H. M. (1917). *Ann. App. Biol.* IV, 91.
- (9) — (1918). *Ibid.* v, No. 2, pp. 102-11.
- (10) — (1921-23). *Bull. Ent. Res.* XII and XIII.
- (11) MULLER, G. W. (1919). *Zs. Wiss. Ins. Biol.* XV, 120.
- (12) PERRIS, E. (1847). Notes sur les Métamorphoses de la *Trichocera annulata* Meig. et de la *Scatopse punctata* Meig. pour servir à l'histoire des Tipulaires. *Ann. Soc. Ent. France*, Sér. II, t. v.
- (13) TONNOIR, A. L. (1926). *Bull. et Annales de la Société Entomologique de Belgique*, LXVI, 353-56.

## REFERENCES TO FIGURES

- Fig. 1. Dorsal view of larva of *Scatopse fuscipes*: (a) antenna; (b) head; (c) prothoracic spiracle; (d) abdominal spiracle; (e) spiracle of 11th segment; (f) projection of 12th segment.
- Fig. 2. Antenna: (a) 3rd segment; (b) spike; (c) 2nd segment; (d) 1st segment.
- Fig. 3. Ventral view of head: (a) mandible; (b) labrum; (c) lateral piece; (d) maxilla; (e) premandible; (f) epipharynx; (g) U-shaped piece; (h) hypopharynx; (i) labium.
- Fig. 4. Labrum ventral view: (a) labrum; (b) lateral or triangular piece; (c) epipharynx; (d) U-shaped piece; (e) premandible.
- Fig. 5. Labrum lateral view: (a) labrum; (b) lateral or triangular piece; (c) epipharynx; (d) U-shaped piece; (e) premandible.
- Fig. 6. Labrum of *S. notata*: (a) labrum; (b) lateral or triangular piece; (c) epipharynx; (d) U-shaped piece; (e) premandible.
- Fig. 7. Right mandible, semi-lateral view.
- Fig. 8. Left mandible, ventral view.
- Fig. 9. Left maxilla: (a) "palp" lobe.
- Fig. 10. Labium: (a<sub>1</sub>, a<sub>2</sub>) hypopharynx, (b) labium.
- Fig. 11. 11th and 12th segments, dorsal view: (a) projection of 12th segment; (b) spiracle; (c) 12th segment; (d) trachea; (e) 11th segment.
- Fig. 12. 11th and 12th segments, ventral view: (a) projection of 12th segment; (b) spiracle; (c) 12th segment; (d) trachea; (e) 11th segment; (f) anus; (h<sub>1</sub>, h<sub>2</sub>, h<sub>3</sub>) anal lobes.
- Fig. 13. Pupa, dorsal view: (a) antennal sheath; (b) prothoracic spiracle; (c) imaginal split; (d) abdominal spiracle.
- Fig. 14. Pupa, ventral view: (a) antennal sheath; (b) labrum; (c) maxillary palp; (d) wing sheath; (e) fore leg; (f) mid leg; (g) hind leg.

(Received April 12th, 1929.)



Fig. 1.

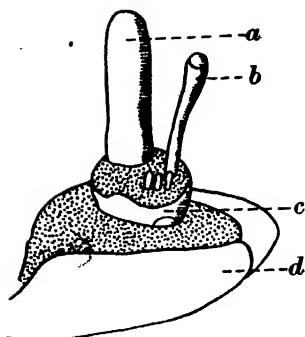


Fig. 2.

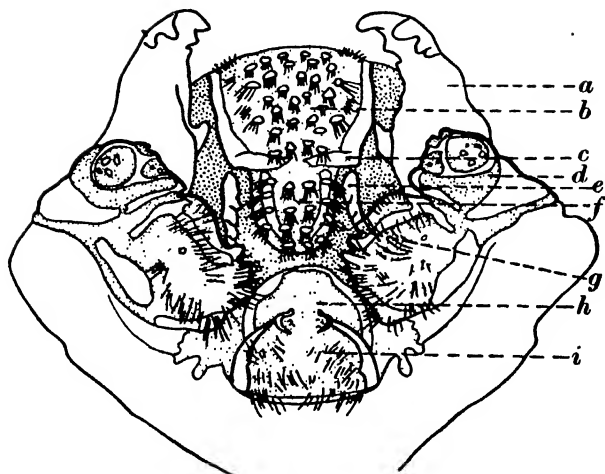


Fig. 3.

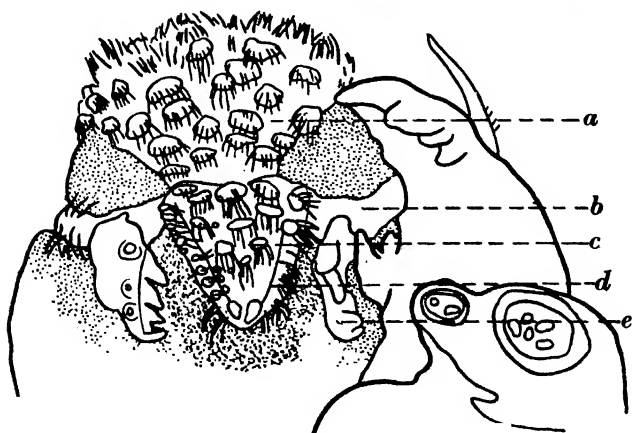


Fig. 4.

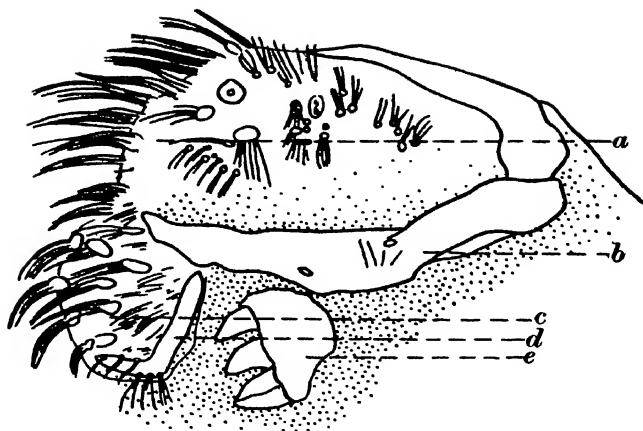


Fig. 5.

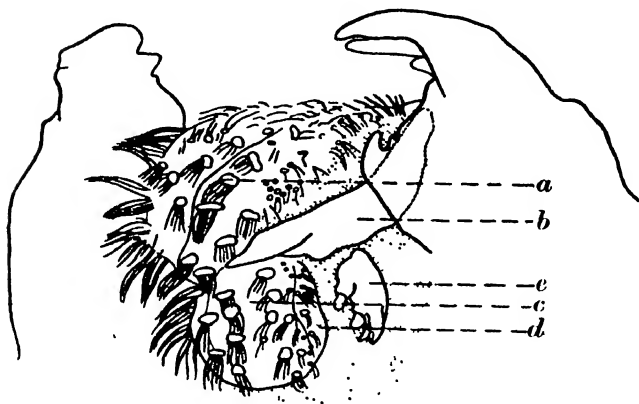


Fig. 6.



Fig. 7.



Fig. 8.

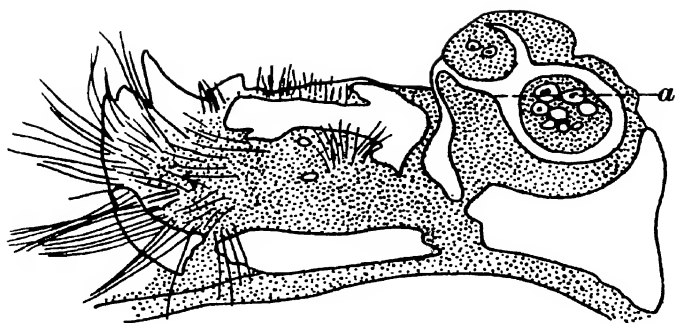


Fig. 9.

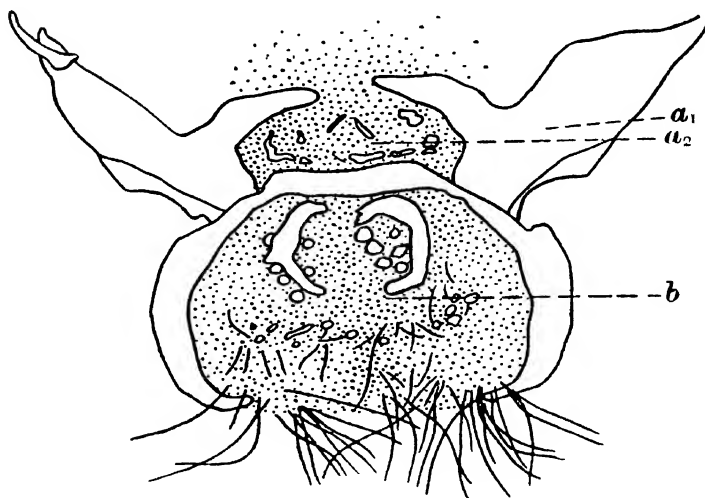


Fig. 10.

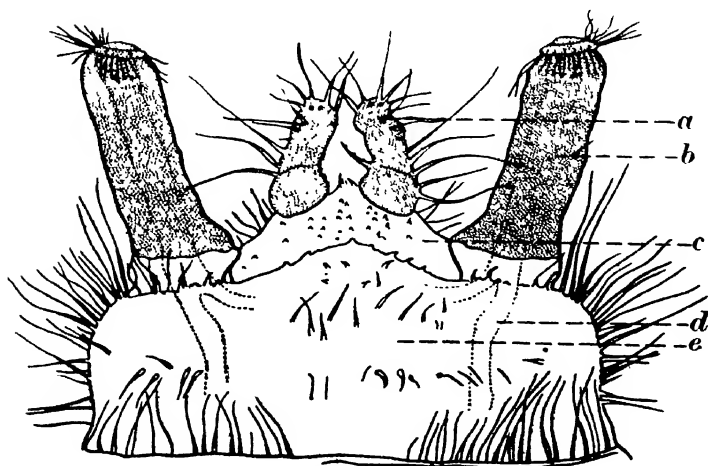


Fig. 11.

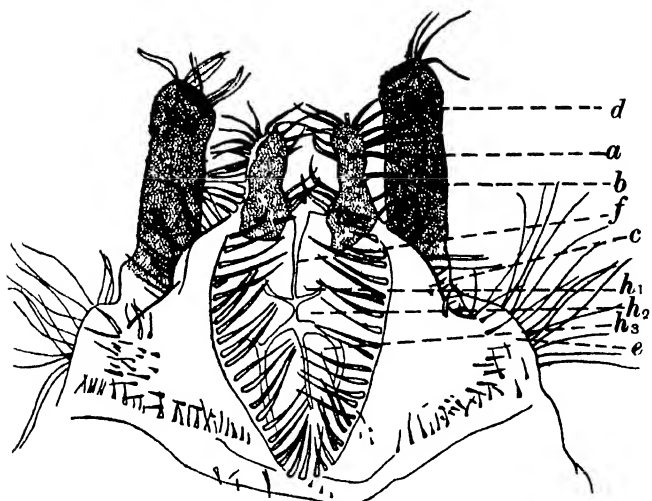


Fig. 12.

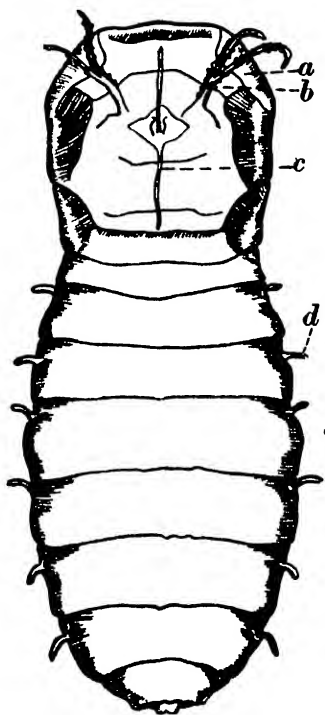


Fig. 13.

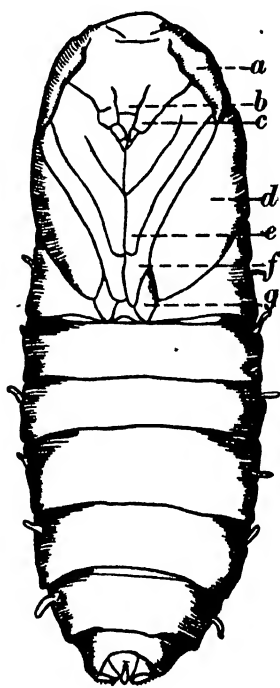


Fig. 14.

## REVIEWS

*Morphological Variation and the Rate of Growth of Bacteria.* By ARTHUR T. HENRICI. Baillière, Tindall and Cox, London, 1928. Pp. xii + 194, with 2 plates, 36 text-figs. and 27 tables. 13s. 6d. net.

The bacteria as individuals of morphological value have received little recognition during the past generation. This has been due largely to the somewhat blind acceptance of the Cohn-Koch dogma of bacterial constancy and simplicity, the acceptance of the culture rather than the single cell as the individual organism and the concentration of attention upon pure cultural technique rather than upon microscopical examination as a method of research. From time to time, however, students have looked at bacterial individuals through a microscope and seen organisms of unexpected shapes and sizes and possessing apparent methods of reproduction differing from those in the authorised teaching. If they ventured to describe these they were told, gently but firmly, that they were dealing with involution forms, contaminations or merely dirty slides, and if they persisted in their lamentable ways they were ostracised as bacteriological bolsheviks, and it was not infrequently hinted that their work was also suspect on other grounds.

A few students, among whom it is not invidious to exalt the name of Löhnis, have, fortunately for bacteriology, had the courage of their observations, and their work is revolutionising the science.

Professor Henrici goes only part of the way with the newer School of pleomorphists and gives an inadequate account of their researches, but, even so, considers that "they have demonstrated beyond question of a doubt that bacteria do regularly show pronounced morphologic variations, the nature and significance of which must be determined before we can make any real progress towards understanding the fundamental biological problems of the Group."

The general theme of the researches in this monograph is given in the following quotations. "This book makes no pretence of being a treatise on the morphology of bacteria, but is rather a record of personal researches undertaken with the hope that by the 'magic of numbers' some order might be brought out of the chaos which has so far filled that field of bacteriology which has to deal with the form and structure of bacterial cells." "In this work I shall show that, contrary to the orthodox teaching, the cells of bacteria are constantly changing in size and form and structure; but that instead of these changes occurring in a haphazard or meaningless fashion, or instead of being phases in a rather vague and complex life cycle, they occur with great regularity and are governed by relatively simple laws which, after more data have been accumulated and analysed, may probably be very precisely formulated." "My investigations indicate that the growth of bacteria in artificial cultures is governed by the same laws as govern the development of a multicellular organism; that their cells during growth pass through exactly the same sort of a development cycle as the cells of a plant or animal, exhibiting in turn an embryonic form during the period of rapid growth, a mature or differentiated form during the period of slow growth or rest, and a senescent form during the period of death; that in short we may speak of a 'cytomorphosis' in populations of free unicellular organisms differing only in degree from that of multicellular individuals."

The author's general conclusion is represented in the following quotation. "My conclusion that the cell changes occurring in cultures of bacteria are a cytomorphosis of the same kind as that exhibited by the cells of a multicellular organism is arrived at only by analogy. No one can state definitely that the growth and cytomorphosis of a population is governed by laws identical with those which govern the growth

and cytomorphosis of a multicellular plant or animal until it has been discovered what those laws are. But the phenomena so exactly parallel each other, no matter from what angle they are viewed, the analogy is so perfect, that we are justified in accepting this theory as at least a working hypothesis for further investigation."

The organisms used in Professor Henrici's studies were *B. megatherium*, *B. coli*, *V. cholerae* and a diphtheroid bacillus. The author's technique is described in detail and the data are fully recorded in numerous text-figures and tables. The volume closes with eight pages of references and author and subject indices; its format is satisfactory and there are few misprints save the word "diphtheroid" the spelling of which is pleasantly varied.

The book is the first of a new series of Monographs edited by Professors Buchanan, Fred and Waksman dealing with the general agricultural and industrial aspects of microbiology.

WILLIAM B. BRIERLEY.

*Die Forleule. Panolis flammea* Schiff. Von Dr HANS SACHTLEBEN.  
Monographien zum Pflanzenschutz. 3. Berlin, Verlag von Julius  
Springer, 1929. Pp. 160, with 35 text-figs. and a coloured plate.  
R.M. 15.80 (paper cover).

In the present year there has appeared the beginning of a new series of handy monographs under the editorship of Dr H. Morstatt which is planned to deal with various aspects of plant-protection. The present part, by Dr Hans Sachtleben, is the third of that series and provides a comprehensive account of the biology and means of controlling the Noctuid moth known in Germany as "die Forleule" and in Britain as the "Pine Beauty." Although common in the British Isles, to as far north as Ross-shire, it is not an insect which occasions serious damage to its host-plant (*Pinus sylvestris*). On the continent, however, its status is very different, for it is a forest pest of notable importance and outbreaks of the insect are frequent. In Germany records of outbreaks have been traced back by Dr Sachtleben for 200 years, and in the chronological list of such "Kalamitaten," which he gives, the first record is in 1725 and the most recent attack lasted from 1921 to 1924. During this latter period wide areas of forest in North and East Germany were infested. During 1925 Dr Sachtleben studied the insect in the State forest of Zossen and also in the laboratory in Berlin, and the present monograph is largely based upon those studies. The moths commence to fly at the end of March and the flight is usually over during May. The eggs are laid singly or in rows on the pine needles, and about 500 are laid by a single female. Until their first ecdysis the young larvae cannot feed upon the previous year's pine needles, and consequently the time of appearance of the young needles has an important bearing upon the incidence of outbreaks of the pest.

Dr Sachtleben's full account of the biology of the insect is followed by a very complete discussion of its parasites and hyperparasites. The economy of these natural enemies has been very fully investigated by him in his 1927 memoir (*Arb. Biol. Reichsanst. Land- u. Forstw.* xv, pp. 437-536), and the gist of the information contained in this work is embodied in the present monograph. A very lengthy catalogue of parasitic Hymenoptera and Diptera is given, and it is noteworthy that the decline of the recent outbreak of the caterpillars of this moth and their final disappearance in July 1925 were due to the extensive parasitization which finally got the upper hand.

With regard to control measures, calcium arsenate and sodium fluosilicate are effective as dusts, and in order to ensure the best results they should be applied early in May when the natural stickiness of the shoots holds the insecticides readily.

The Chalcid parasites *Trichogramma evanescens* and *Pteromalus alboannulatus*, which affect the eggs and pupae respectively, are not destroyed by the dusting and consequently these valuable natural controlling agents merit attention. They are easy to maintain by breeding, and the recent work by Flanders in America, on the artificial propagation of the first-mentioned parasite, is referred to in this connection.



In addition to insect enemies, insectivorous birds and mammals also come in for consideration, and the monograph represents a very complete ecological study of the insect from diverse points of view.

Like most German scientific works, it is admirably printed and the coloured plate of the life-history of the insect is well executed: the text-figures are not numerous but will probably be found sufficient for their purpose, while at the end of the work there is a very full bibliography of the insect and its parasites.

A. D. IMMS.

*Die Rubenblattwanze Piesma quadrata* Fieb. By JOH. WILLE. Monographien zum Pflanzenschutz. 2. Berlin, 1929. Pp. 116. R.M. 9.60.

Sugar-beet has been grown commercially in Germany since about 1800, and is now among the more important crops. For 100 years it was not invaded by the Lace Bug *Piesma quadrata* Fieb, although this insect was well known to occur on many of the wild Chenopodiaceae as well as on other plants, e.g. in a survey of the pests of the sugar-beet made in 1882 *Piesma* was not recorded, and it has a range in Europe below the 600 m. level from Russia to Great Britain and the Alps to Scandinavia. About the beginning of this century, however, it began to attack the sugar-beet, and, although in some districts it is still chiefly confined to its old hosts, the beet-invading habit has spread over a large area, especially in the middle and east of Germany, and is still spreading. In itself *Piesma* does relatively little damage to the plant, unless the infestation is exceptionally heavy and at the same time the invaded plant young and tender. But its adoption of the new host has been followed by the appearance of a new disease in the sugar-beet, the Krausel Krankheit, or leaf-curl disease, which is a serious menace to the industry in the areas in which it has appeared, and from which it is spreading with considerable rapidity. This is a virus disease, similar in many respects to but not, it seems, identical with the curly-top disease of sugar-beet found in the United States of America. It is carried by *Piesma quadrata*, and, so far as is yet known, by this insect only, and it is transmissible to a number of other plants closely related to the sugar-beet.

In this monograph these points are discussed in detail by Dr Wille, and full details are given of the morphology and life history of the insect, the symptoms of the disease, and the measures to be taken to control its incidence. So far, the most successful method has been to plant a catch-strip of sugar-beet round the fields about two weeks before the main crop is sown. This catch-strip, developing a fortnight before the main crop, is invaded by the *Piesma* that have survived the winter, and, at the time the main crop is beginning to appear, this strip is disinfected thoroughly, and then buried deep in the soil. The results of this procedure are said to be very encouraging.

J. HENDERSON SMITH.

CAMBRIDGE: PRINTED BY  
W. LEWIS, M.A.  
AT THE UNIVERSITY PRESS





L. A. R. I. 75.

IMPERIAL AGRICULTURAL RESEARCH  
INSTITUTE LIBRARY  
NEW DELHI.

Date of issue.	Date of issue.	Date of issue.
18-10-38	2-2-53	
19-12-38	2 NOV 1953	
28-7-35	<del>28-7-35</del>	
7-2-46	27-8-53	
26-9-40	16-5-53	
10-1-41	1-7-12-53	
17-1-42	7-5-65	
16-1-43	28-1-66	
8.9.43	22.2.66	
9.11.43		
13.11.43		
6.3-46		
17.2.48		
17.6.52		